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EDITED BY

E. RAY LANKESTER, M.A., LL.D., F.R.S.,
Fellow of Exeter College, Oxford, and Jodrell Professor of Zoology in University College, London;

WITH THE CO-OPERATION OF

W. T. THISELTON DYER, M.A., C.M.G., F.R.S.,
Assistant Director of the Royal Gardens, Kew;

E. KLEIN, M.D., F.R.S.,
Joint-Lecturer on General Anatomy and Physiology in the Medical School of St. Bartholomew's Hospital, London;

H. N. MOSELEY, M.A., LL.D., F.R.S.,
Linacre Professor of Human and Comparative Anatomy in the University of Oxford,

AND

ADAM SEDGWICK, M.A., F.R.S.,
Fellow and Assistant-Lecturer of Trinity College, Cambridge.

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On the Relations of the Yolk to the Gastrula in Teleosteans, and in other Vertebrate Types.

By

J. T. Cunningham, B.A.,
Fellow of University College, Oxford; Superintendent of the Scottish Marine Station.

With Plates I, II, III, and IV.

The study of the ova of marine Teleostean fishes is now being actively pursued by many naturalists and is yielding results which are, in the present stage of the progress of zoological science, of great importance. These results are of various kinds. Some of them consist in the discovery of the conditions which affect the life and development of different kinds of ova, and the invention of methods and apparatus by which the favorable conditions can be most efficiently produced in the biological laboratory or in the premises of the pisciculturist. To this class of results the present paper makes but little contribution. Another class comprises the descriptions, measurements, and illustrations which set forth the peculiarities characterising the eggs of the several species. The knowledge of these peculiarities in the ovum of a given species is an addition to the sum of known facts concerning the life-history of that species; and the accurate observation of these peculiarities is of special value in the case of pelagic ova, because in these inconspicuous features afford the only criteria by which the species to which they belong can be ascertained. To this second class of results some slight additions are made

in this paper. A third class comprises discoveries of new facts concerning the processes of Teleostean development; explanations, more or less hypothetical, of the steps by which that process of development has been produced by the contending influences of heredity and adaptation; and attempts to draw from the facts ascertained concerning Teleosteans, inferences as to the significance of the features of development peculiar to other vertebrate types. To suggest such explanations, of some of the earlier stages of Teleostean development, and to draw some inferences of the kind I refer to, are the principal objects of my paper.

Part I.—Practical and Speciegraphical.

The facts and illustrations concerning the development of the herring which are contained in this paper are the result of a study of the development of that species made last year, and which has been described in a former paper (13). I first fertilised ova from the haddock (Gadus æglefinus, L.) on March 11th of the present year, off the west side of the May Island, on board a line-fishing boat belonging to Anstruther. The eggs and milt were simply squeezed into a large bottle of sea-water and the eggs were not disturbed until after they had been taken ashore. I made observations on these eggs in the living condition at Anstruther, at the fish-curing establishment of Mr. David Murray, who very kindly placed at my disposal all the accommodation for my work which his premises afforded. The number of eggs which I obtained on this occasion was small and they all died before hatching. On March 30th I obtained another supply of ova from the haddock, and also a number of those of the whiting, G. merlangus, L., and cod, G. morrhua, L., on board a steam-trawler, which was working a few miles to the east of the Isle of May. The eggs were treated in exactly the same way as the haddock eggs on the former occasion. I tried to obtain eggs from several other species of fish which came up in the trawl, but was unsuccessful, except in the case of the three species mentioned; Lophius

piscatorius was plentiful but not ripe; Trigla gurnardus, Pleuronectes limanda, P. microcephalus, and Hippoglossoides limandoides were also unripe; Pleuronectes platessa was spent.

The fertilised eggs were brought to the Marine Station on the morning of March 31st, and transferred to clean water in a number of vessels. The eggs were removed by skimming them with a spoon from the surface of the water, where they floated when undisturbed. They still floated at the surface when placed in the sea-water of the station, although its density is less than that at the Isle of May; the sp. gr. of the latter at 15·6° C. as compared with pure water at 4° C. is 1·025, of the former 1·023. The water in which the eggs were was changed from time to time, usually once in two days; this was sometimes effected by carefully siphoning off the water without disturbing the eggs at the surface, and then replenishing the vessels with clean water. Sometimes, when there were a large number of dead eggs at the bottom of a vessel the living ones were transferred by means of a spoon to another vessel full of clean water. One of the vessels was aërated with a stream of air-bubbles from a small aërating apparatus, but the eggs in this did not thrive any better than those treated solely by the method of transference.

On April 3rd, in addition to a new supply of the three kinds of ova before obtained, some eggs of the grey gurnard (Trigla gurnardus, L.) were brought to me. These floated at the surface of the water in which they were carried, and were treated in the same way as the others, but they immediately sank to the bottom when placed in the water of the station. Thus their specific gravity is greater than that of the station water and less than that of the sea water at the mouth of the Forth. It lies between 1·023 and 1·025, while that of the ova of the three species of Gadus is less than 1·023.

Temperature to which the Ova were exposed and Rate of Development.

The ova of the whiting and haddock obtained on March 30th were hatched at the Marine Station. The temperature of the water in which the eggs were kept varied from 6° C. to 9° C., average 7·5° C. The temperature of the sea outside the Isle of May at the time the eggs were taken was almost constant at 6·1° C. The whiting hatched on the eleventh day, haddock on the twelfth. Some were exposed after seven days to a somewhat higher temperature, the thermometer sometimes for a short time being at 13° C., and of these the whiting began to hatch on the tenth day, the haddock on the eleventh. Some of these larval whiting were kept alive eleven days after hatching in a shallow dish, a piece of Enteromerpha being placed in the water with them as a source of oxygen. The cod eggs obtained March 30th all died before hatching. A few of the cod eggs obtained on April 3rd, at about the same temperature as before, hatched on April 15th, the twelfth day. It is not possible to judge accurately from these few data how long the ova take to develope in the conditions obtaining in the sea outside the Isle of May. The haddock and cod evidently require the same time to reach the hatching stage, and the whiting a little less time than these. Probably at the temperature of 6° C. to 7° C. obtaining in the month of March at the mouth of the Firth of Forth, the cod and haddock would hatch about the sixteenth day after fertilisation. Professor Ryder (3) gives twenty-one days as the time occupied in development by the cod eggs studied by him, but he does not mention the temperature to which they were exposed.

Description of Ova.

The fertilised ova of the cod, haddock, and whiting are in all respects similar to one another except in size. The sizes of the ova mentioned in this paper are :

G. merlangus . . .	1·25 mm. diameter.
G. morrhua . . .	1·39 ,, ,,
G. æglefinus . . .	1·45 ,, ,,
Trigla gurnardus . .	1·60 ,, ,,
Clupea harengus . .	·95 ,, ,,

The measurements of the first four were obtained by measuring the drawings of them made by means of Abbé's camera lucida, always with the same combination of lenses in the microscope. The measurements so obtained are, perhaps, not absolutely accurate, but the errors are about the same in all cases. The ovum suffers probably some slight alteration in shape, due to its own weight, when resting on a glass slide in a small quantity of water, which were the conditions under which the drawings were made. The ova, too, of one species, vary slightly in size; but the above measurements are at least approximately correct. The diameter of the herring egg given is taken from Kupffer.

The fertilised ovum of each of the three species of Gadus mentioned consists, soon after fertilisation, of a sphere of perfectly transparent, structureless, colourless yolk, at one pole of which is a mass of protoplasm forming the blastodisc. The blastodisc projects slightly beyond the regular contour of the sphere, and has a peculiar colour, resembling a light shade of what is known as terra-cotta colour. This colour remains during the process of simple segmentation, but it gradually gets lighter, and disappears at the time when the segmentation cavity is formed. After this period the protoplasm of the embryo is colourless, and only a little less transparent than the yolk mass. The ovum is enclosed in a vitelline membrane, which is thicker in the cod than in the other species, and in which no structure is to be observed with a power of forty diameters. Between the ovum and the vitelline membrane is a space filled with homogeneous fluid, chiefly, no doubt, sea water. This "perivitelline" space remains throughout the whole development without varying in size. In the ova of Gadus it is not very large, but I have seen other ova taken in the tow-nets in which the diameter of the perivitelline space at its widest part was equal to

the diameter of the ovum. The above description, founded on my own observations, agrees very closely with the account given of the cod's ovum by Professor John A. Ryder.

The ovum of Trigla gurnardus is distinguished by the presence of a single large oil-globule, of brownish-yellow colour, which is situated between the surface of the yolk and the perivitelline membrane. The most surprising fact about this oil-globule is that it is capable of free motion in the perivitelline space. When the egg is rotated on a slide the oil-globule rapidly rises to the highest pole in whatever position the egg is placed. It does this even when the segmenting blastodisc is placed uppermost, passing between the blastodisc and the vitelline membrane. Whether the oil-globule is at a later stage enveloped by the blastoderm I am not able to say. I am not aware that a moveable oil-globule has been described in any Teleostean ovum before, and I cannot say at present whether the peculiarity is confined to the eggs of the gurnard or not.

The larvæ of the whiting and haddock when hatched are in a condition similar to that of the newly-hatched cod as described by Ryder. The intestine ends immediately behind the yolk, and if it is open at all does not open at the edge of the ventral median fin; the terminal portion of the intestine reaches only half way down the breadth of that fin. There are no red blood-corpuscles, but the eyes are pigmented, and there are scattered stellate pigment-cells on the back and sides. The mouth is not formed, but the gill-slits are opened. The yolk is still of considerable size, and by its buoyancy causes the young fish to be suspended in the water back downwards.

Part II.—Embryological.

Some time after fertilisation in a Teleostean a furrow is formed across the blastodisc (fig. 1), which is at first superficial, but is soon continued by a plane of division to the lower limit of the disc. At right angles to the first a second furrow and plane of division are next formed, so that the blastodisc is divided into four equal and similar parts, in each of which

there is a nucleus. By the continued repetition of this segmentation, which soon begins to take place also in planes parallel to the surface, the blastodisc is converted into a mass of nucleated cells, forming the blastoderm. While the cells are still large enough to be distinguished by a power of forty diameters the blastoderm in the ova of the cod, haddock, and whiting is a prominent hemispherical projection having a slightly convex base, which rests in a shallow concavity of the yolk. The appearance of the living ovum of the haddock at this stage is shown in fig. 2, which represents an optical section in a plane dividing the ovum symmetrically. Fig. 11 represents the corresponding stage in the ovum of the cod observed in a similar position. If a view is obtained of a part of the surface of the ovum, which includes part of the edge of the blastoderm, it is seen that the boundary between the latter and the yolk is not sharply defined; that the cells at the edge of the blastoderm have no planes of division separating them from the yolk-mass, though their nuclei and the planes of division separating them from one another and from the other cells of the blastoderm can be distinctly seen. The appearance referred to is shown in fig. 12 as seen in the ovum of the cod. Agassiz and Whitman (1) have ascertained from sections of the ovum of Ctenolabrus that the cells at the edge of the blastoderm are at the sixteen-cell stage, and from that period up to the period of invagination continuous with a layer of protoplasm, which extends over the yolk outwards beyond the edge of the blastoderm, and inwards beneath the blastoderm. According to their results the blastoderm is connected with the yolk only by the circular series of cells at its edge, the rest of it being separated from the yolk by the segmentation cavity, which is present as early as the sixteen-cell stage, but remains for some time very small.

The state of things described above, as seen in the living ovum of the cod, agrees perfectly with the results of Agassiz and Whitman. By careful focussing the protoplasm covering the yolk in the neighbourhood of the blastoderm can be seen at this and earlier stages of segmentation in optical sections,

especially close to the edge of the blastoderm where the layer is thickest. Study of the living ova does not enable one to see the segmentation cavity or any separation between the centre of the blastoderm and the yolk at the early stages now under consideration, but there can be no doubt that Agassiz and Whitman have given the true history of the origin of the periblast. The history of the relations of the yolk to the blastoderm during the period of segmentation may from their results, and from what can be seen in living pelagic ova, be read as follows: —When the blastodisc is formed it is not completely separated from the yolk, although its limits appear to be so distinct, it remains continuous with protoplasm in the yolk-mass, protoplasm of which the greater part forms a thin superficial layer extending from the edge of the blastodisc over the surface of the yolk. When the blastodisc divides into two parts, each of these is to be considered as being continuous with half the yolk through the yolk protoplasm. When the blastoderm consists of sixteen cells the twelve peripheral at least of these are still continuous with the yolk protoplasm. The cells at the edge of the blastoderm remain during segmentation continuous with the yolk protoplasm, and at all stages the yolk-mass may be considered as potentially divided into a number of portions, each belonging to one of these cells.

As is seen in fig. 12, during the process of segmentation the division of a nucleus at the edge of the blastoderm is accompanied by the formation of one plane of cell division parallel to the edge of the blastoderm between the outer daughter nucleus and the blastoderm, and of other planes perpendicular to the edge of the blastoderm, while there is no plane of division between the outer daughter nucleus and the yolk. When the stage at which invagination begins is approached the formation of planes of division perpendicular to the edge of the blastoderm ceases; thus the outer row of nuclei are separated by cell-division planes from the rest of the blastoderm, but not from one another. After this these nuclei continue to divide, but no planes of division are formed to divide the protoplasm in which they are situated into corresponding cells. These nuclei

are from this stage onwards the nuclei of the periblast, and, as shown by the researches of Agassiz and Whitman, they multiply in two directions—outwards from the edge of the blastoderm over the uncovered yolk, and inwards beneath the blastoderm. Whether the continuity between the nucleated periblast and the cellular blastoderm is as completely broken as I have now stated, or at later stages cells are formed from the periblast which are added to the blastoderm, is a question which will be discussed further on. It is certain, at all events, that the nuclei in the periblast are at first confined to that part of it which is beneath the blastoderm or near its outer edge, there are none at the lower pole of the yolk. In sections which are in my possession of the herring ovum at the stage when the blastoderm has enveloped a little more than half the yolk, the nuclei of the periblast are still confined to the neighbourhood of the edge of the blastoderm, and do not extend to the pole of the uncovered part of the yolk.

There can be little doubt that the origin of the so-called free nuclei in Elasmobranchs and Sauropsida is exactly similar to that which is now demonstrated for the nuclei of the periblast in Teleostei. It was at first supposed that the yolk nuclei in those Vertebrates in which the yolk is not cellular arose spontaneously, a supposition which Balfour in his 'Comp. Embr.' considered improbable. A new and surprising proposition concerning the origin of the nuclei of the Teleostean periblast has recently been sustained by H. Hoffmann (11), namely, that the first nuclear division after fertilisation takes place in a plane parallel to the surface of the blastodisc, that the upper of the daughter nuclei is the parent of all the nuclei of the blastoderm, and the lower the parent of all the nuclei of the periblast. The results of Agassiz and Whitman are in direct opposition to those of Hoffmann, and the conclusions of the former are based on very strong evidence, while the existence of the state of things represented in fig. 12 is altogether incompatible with Hoffmann's views.

If it be admitted that the nuclei in the Teleostean periblast, as well as the yolk nuclei in Elasmobranchs and Sauropsida,

arise in the way that has been described above, it is possible to trace the steps by which the modifications of the segmentation process exhibited by different vertebrate types have been reached. There has never been any difficulty in understanding how the unequal segmentation of the Amphibian ovum has been derived from the primitive regular segmentation; the presence of a certain amount of food yolk, which at a very early stage of segmentation is confined to the lower cells of the blastosphere, has caused these cells to be many times larger than the upper cells. The same is the case in the ovum of Petromyzon. In Lepidosteus and Acipenser (*vide* Balfour, 'Comp. Embr.,' vol. ii) we have the next modification. Some of the cells into which the yolk-containing portion of the blastosphere is divided are in these forms distinctly defined, but much larger than the corresponding cells in the Amphibian; and others of the yolk-cells are incompletely separated from one another. In Teleostei, Elasmobranchii, and Sauropsida the separation of the yolk-cells from one another has ceased altogether, and they form a syncytium. But in all cases the process of segmentation is essentially the same, and the first step in the process is not the separation of a yolk-containing cell from a purely protoplasmic cell, but the division of the ovum into two similar cells, each containing a cap of protoplasm and a large quantity of food-material.

At the end of what may be called the period of simple segmentation the blastoderm in the ovum of the cod or haddock, has the form of a doubly convex lens resting in a shallow concavity of the yolk (figs. 3, 13). A thickened ring of periblast at the edge of the blastoderm is usually, as shown in fig. 13, conspicuous at this stage in optical sections, and it was this appearance of the periblast which first attracted attention to that layer.

The Process of Invagination.

The bi-convex blastoderm just described does not long retain its shape; it begins to extend laterally and to thin out at the centre. At the same time the central part raises itself up from the yolk, thus forming a closed cavity, the segmentation cavity.

This stage is illustrated in figs. 4 and 14, the latter representing simply an optical section, while the former gives also a projection of the part of the blastoderm between the eye and the plane focussed. Fig. 15 shows a surface view of the blastoderm at the stage in question. The form of the blastoderm is now no longer symmetrical; in the neighbourhood of one radius from its centre it is considerably thicker than elsewhere, and this thickened part is the first indication of the embryo. It will be seen, when the history of the blastoderm is traced on to its later stages, that the part of the embryo which is thus early formed is the dorsal region of the head.

Soon after the first appearance of the segmentation cavity a distinct layer of cells is seen extending inwards from the edge of the blastoderm towards the centre. This layer is in contact with the periblast below and with the blastoderm above; it is continuous externally with the upper layer of the blastoderm, and thus appears to be formed by a growth inwards of the edge of the blastoderm. The relations of this layer, as seen in living ova, are shown in figs. 5 and 16. The invaginated layer, as it is usually called, does not extend over the floor of the segmentation cavity, but is confined to the peripheral region of the blastoderm. For this region the name embryonic ring may be conveniently used. The segmentation cavity maintains the same relations as at the previous stage, except that it is somewhat flattened, and more eccentric in position: the whole blastoderm has extended and covers a larger area of the yolk than before. The embryonic thickening has become longer and more distinct. The invaginated layer extends further inwards in the neighbourhood of the embryonic rudiment than beneath the rest of the edge of the blastoderm. In fig. 17 is represented the appearance of the surface of the blastoderm after the formation of the invaginated layer: the lighter region marks the position and extent of the segmentation cavity, and the darker, the area occupied by the invaginated layer. The embryonic rudiment forms a blunt projection inwards towards the segmentation cavity, while the outer contour of the blastoderm is regularly circular.

In the memoir already referred to Ryder gives two figures (3, figs. 15 and 16) illustrating the period of development now in question, as studied by him in the cod. One of these figures (loc. cit., fig. 15), representing an optical section, indicates a state of things which, if my present views are well founded, is impossible. According to this figure the segmentation cavity is completely bounded below by a layer of "hypoblast," continuous, apparently, with what I have called the invaginated layer; but the limits of the invaginated layer, clear and distinct as they are in the living ovum, are not indicated in Ryder's figure.

I have never seen any stage in which the segmentation cavity was bounded inferiorly by a layer of cells or by anything but the surface of the yolk (periblast); and if there be such a stage at all it certainly does not occur after the appearance of the invaginated layer; but Ryder's figure evidently belongs to the same stage as that represented by Nos. 5 and 16 of my figures, and therefore if my figures are correct his is erroneous.

In the text of his paper (p. 38) Ryder contradicts the proposition implied in his figure, that the segmentation cavity is bounded inferiorly by a stratum of distinct cells, and says that its floor is formed "by the yolk hypoblast, which is not truly hypoblastic, and which corresponds to the granular layer of Balfour." It is difficult to form a clear conception from Ryder's paper of what he has seen of the relations of the primary layers at this period of Teleostean development, or what conclusions he has drawn. The figures he gives of the relations of parts at succeeding stages agree closely with my own results, but all that he says distinctly concerning the early condition of the invaginated layer is that it arises by delamination from the blastoderm.

There are two subjects of inquiry connected with the segmentation cavity and the invaginated layer, which in Ryder's pages are continually confused. Firstly, what are their relations with other parts of the blastoderm at successive stages? secondly, what are the processes by which those relations are produced?

Ryder refers to Haeckel's account of the development in a

pelagic Teleostean ovum (4), in order to contradict several of the propositions contained therein; but the propositions of Haeckel which he refutes are not those which are altogether erroneous. Ryder says that Haeckel is wrong when he implies that the whole under surface of the blastoderm is lifted up from the yolk, and remains in contact with the latter only round its margin; and yet two pages before he expresses agreement with Klein in the view that the segmentation cavity is formed by the elevation of the blastoderm, so that it is freed from contact with the parablast layer lying just below it. The second proposition of Haeckel's which Ryder disputes is that the hypoblast is formed by a reflection inwards of the margin of the blastoderm; and, thirdly, he denies the statement of Haeckel, that the segmentation cavity disappears.

With regard to this last point it is certainly true that in the cod and haddock, and probably in all pelagic Teleostean ova, the segmentation cavity is not obliterated, as Haeckel taught, immediately on the formation of the invaginated layer, by that layer growing inwards till it forms a complete stratum beneath the blastoderm. The segmentation cavity persists, as will be seen below, till the yolk is almost completely enveloped by the blastoderm; but whether it persists in the adult, as Ryder supposes, is another question. According to my own observations, Haeckel was right in his account of the formation of the segmentation cavity, and of the earliest relations of the invaginated layer, except that he was not aware of the existence of the periblast.

The relations of the invaginated layer at the earlier stages of its existence have been correctly represented by Kingsley and Conn, as seen in the ovum of Ctenolabrus cœruleus, in figs. 22 and 23 of their memoir (2). The relations of the segmentation cavity at later stages are not clearly indicated by these authors.

As far as can be judged from their paper the conclusions of Agassiz and Whitman (1), whose observations were made on Ctenolabrus, Pseudorhombus melanogaster, Ps. oblongus and Tautoga, agree with my own as to the relations

of the invaginated layer or ring, but their attention is chiefly devoted to the method of its formation, which is now to be considered more particularly.

Haekel's view (4) was that the invaginated layer was produced simply by the multiplication of the cells at the edge of the blastoderm, the new cells arranging themselves as a single layer, which insinuated itself between the blastoderm and the yolk. Ryder, as has been mentioned above, believes that there is no ingrowth at all from the edge of the blastoderm, but that after the appearance of the segmentation cavity the cells of the blastoderm split up *in sitû* into three layers, the lowest of which, next to the periblast, comprises hypoblast and mesoblast; the next above is the sensory layer of the epiblast, and the outermost is the epithelial or epidermic layer. Kingsley and Conn formulate very distinctly the conclusion, supported by detailed evidence, that in the ovum of *Ctenolabrus cœruleus* the invaginated layer is produced, as Haeckel believed, by the gradual ingrowth of the edge of the outermost layer of the blastoderm. According to these observers the layer consists of only a single stratum of cells. Agassiz and Whitman are also of opinion that the invaginated layer arises by ingrowth from the edge of the blastoderm; "though, of course," they say, "there is no such wholesale invagination as supposed by Haeckel." This means, apparently, that the invaginated layer does not, as Haeckel supposed, extend completely beneath the segmentation cavity. These authors regard the centrepetal multiplication of periblast nuclei beneath the blastoderm as part of the process of invagination. They also make two statements which require to be considered: firstly, that the invaginated layer is continuous at the edge of the blastoderm, not with its outermost layer, but with the layers beneath; and secondly, that the invaginated layer is a single stratum in all regions of the ring except beneath the axis of the embryonic rudiment, where it is two to four cells deep. The former of these statements cannot be regarded as proved by the figure of a prepared section, to which the authors refer as evidence. The interpretation of the second statement is

probably that beneath the axis of the embryonic rudiment the hypoblast is continuous with cells destined to form the notochord.

The evidence of the actual ingrowth of a cell layer from the edge of the blastoderm is not very clear in the case of the salmon, to judge from the elaborate investigations concerning that form which are described in published memoirs (12). But it is certain that in ova of the Salmonidæ the general relations of the embryonic rudiment, germinal ring, and segmentation cavity are the same as in pelagic ova, and the lowest cells of the thickened rim of the blastoderm are continuous at the edge with the epiblast, although there is no single stratum distinctly marked off as an invaginated layer. It is possible that the layer in question, the lowest layer of the germinal ring, may vary in its mode of origin; in pelagic ova, such as Ctenolabrus, cod, and haddock arising chiefly by centripetal multiplication of cells, in Salmonidæ to a great extent by delamination from the blastoderm. The latter mode of formation can easily be explained by the familiar principle of abbreviation of development, according to which cells attain a position ontogenetically during segmentation, which phylogenetically was only reached by actual change of position.

A third and quite novel view concerning the origin of the invaginated layer has been recently brought forward by George Brook (13), who believes that in Trachinus vipera the layer is produced by the formation of distinct cells from the nucleated protoplasm of the periblast. I am not able to subject this proposition to criticism any further than to say that the appearances in the living ova of the haddock and cod are best explained in the view that the layer in question arises by a centripetal ingrowth of cells from the edge of the blastoderm.

In whatever way the invaginated layer is produced there is no uncertainty concerning the main relations of the parts in the ova of the cod and haddock at the stage represented in figs. 5, 16, and 17. The yolk is a nearly spherical mass, of which that part beneath the blastoderm and a little beyond its edge is provided with a layer of nucleated protoplasm—the periblast.

The blastoderm has a circular form, and beneath its edge is a layer of cells in the form of a flat ring, whose lower side is in contact with the periblast. In the neighbourhood of one of its radii the blastoderm is much thicker than elsewhere, and this thickening forms the embryonic rudiment, beneath which the invaginated layer extends further inwards than at other parts of the ring. Where the invaginated layer is absent there is a cavity separating the more central part of the blastoderm from the periblast—the segmentation cavity. It is a conspicuous and important fact that the inner edge of the invaginated layer is distinct and sharply defined, and that there is apparently no direct continuity between this edge and the periblast on which it rests. The significance of this fact will be considered subsequently.

Growth of the Blastoderm round the Yolk.

Successive stages in this process, as seen in the living ovum of the haddock, are represented in figs. 6—9; fig. 18 shows a late stage in the process in the whiting; figs. 20, 21, and 22 represent successive stages in the herring ovum. Some stages of the process in the cod ovum are correctly represented by the figures of Ryder; in Ctenolabrus by the figures of Kingsley and Conn. The main features of the process as seen in the haddock and cod are that the embryonic rudiment grows in length with the growth of the blastoderm, its posterior end always remaining at the edge of the latter; that the segmentation cavity increases greatly in extent, at the same time its depth decreases, and it becomes very thin; that the part of the embryonic ring outside the embryonic rudiment decreases slightly in breadth, and that the ring gradually contracts as it reaches the lower pole of the ovum, and diminishes to a small opening on the posterior end of the dorsal surface of the embryo, and this opening finally closes. The most important fact about the envelopment of the yolk by the blastoderm is that the embryonic ring is gradually absorbed by the increasing embryonic rudiment until the whole of it forms part of the dorsal region of the embryo. As this process of absorption goes on the

invaginated layer beneath the axis of the embryonic rudiment increases at the expense of the invaginated layer of the embryonic ring, the rest of which goes to form the external part of the embryonic rudiment. The whole of the embryonic ring thus belongs to, and is formed into, the dorsal region of the embryo. These phenomena are discussed at greater length in a subsequent section. The segmentation cavity is seen at the stages represented in fig. 6 and 7, both superficially, and in optical section at the side of the ovum opposite to the embryo. At the stage shown in fig. 8 it is no longer visible in section, though the line *s s* marking the extent of the invaginated layer is still distinct. In fig. 9 the line *s s* is not seen, and at this stage the segmentation cavity is not visible. As far as can be judged from my observations the segmentation cavity is obliterated at the stage represented in fig. 8 by its epiblastic roof coming into contact with the periblast. Before the yolk is completely enveloped by the blastoderm the differentiation of organs has begun to take place in the embryo. The cerebral part of the eye is visible at the stage shown in fig. 7, and at the time the envelopment is complete the separation of the mesoblastic somites has commenced. It will be seen that, on the supposition that the anterior end of the embryonic rudiment is a fixed point in relation to the yolk, the embryonic side of the blastoderm at first grows faster than the opposite side; the distance from x to z in figs. 6, 7 is less than the distance from x to y. In fig. 8 the contrary is the case. After a certain time the growth in length of the embryo takes place more slowly, and at the time when the embryo is completely enveloped the embryo in the haddock and whiting occupies half a meridian of the yolk. In the herring ovum (fig. 25) the embryo at the corresponding stage is longer in proportion to the yolk. The herring ovum is far from being as transparent as are pelagic ova, and in consequence of this in the former the relations of the internal parts cannot be distinguished in the living condition; thus in fig. 23 the limits of the segmentation cavity and invaginated layer are not distinct. From my sections of ova at the stage shown in

fig. 23 I have not been able to obtain much more information than from living material, and I am inclined to think that, while the difficulty of preserving and preparing good sections of herring ova may be partly the cause of the obscurity, in that species the segmentation cavity is not developed to the same extent, nor the invaginated layer so distinctly defined, as in pelagic ova.

Significance of the earlier features of Teleostean Development.

In his "Gastraea-theorie" Haeckel attempted to explain the relation of the process of development in a Teleostean ovum studied by himself, to the process of development in a typical gastrula (4). The eggs on which his observations were made were pelagic and were taken by means of the tow-net, near Ajaccio in Corsica; they were ·64 ·66 mm. in diameter, adhered to one another in small masses, and each possessed a single large oil globule at the nutritive pole. Haeckel supposed these eggs to belong to the genus Lota or some other genus among the Gadidæ allied to Lota. I do not know if it has ever been ascertained to what species the eggs he describes actually belong: the eggs of the genus Gadus described in the present paper are all destitute of oil globules, but it does not necessarily follow that such structures are wanting in the eggs of all the Gadidæ.

In Haeckel's account of the development, the existence of the layer now known as periblast was denied altogether. He declared that by the evidence of his Teleostean eggs the false parablast theory of His, and all similar theories, according to which cells arose in the yolk and afterwards took part in the formation of the embryo independently of the two primary germinal layers, were completely refuted. The parablast theory of His was certainly not the final explanation of the significance of yolk-nuclei, but it is curious that Haeckel should have adopted a view concerning the yolk-mass which was inconsistent with his fundamental proposition that the egg, no matter how much yolk it might contain, was a single

cell. He regarded the yolk as a quantity of passive material which was contained in the gastrula cavity, projected from the blastopore, and was ultimately enveloped entirely by the hypoblast. But since the egg before segmentation is a single cell, no portion of it can be separated from the rest except by a process of cell-division, and any such separated portion must have the value either of a cell or a number of cells. It remains true, as shown below, that the yolk projects from the blastopore; but the relations of yolk and blastopore to one another and to the embryo were not by any means completely or correctly stated in Haeckel's account. These relations need to be examined again in the light of the additional knowledge which has been gained since the period of the "Gastraea-theorie."

The facts from which an inquiry into the ancestral origin of the processes of Teleostean development must start are—firstly, that in the actual development of many animal forms the primitive digestive cavity is formed by an invagination of part of the wall in a hollow spherical body; secondly, that in Elasmobranchii and Amphibia an inflection of, or growth inwards from, the edge of the blastoderm forms a layer beneath the axis of the embryonic rudiment, which layer becomes without material alterations in its constitution the dorsal wall of the permanent intestine.

The broad proposition that this process of inflection in Elasmobranchs and Amphibians represents the primitive process of invagination in a simple gastrula is universally accepted by embryologists. There is still a good deal of obscurity about the history of the successive changes in the development of the embryo by which the modified invagination in the two types mentioned was derived from the primitive invagination in a typical gastrula.

Without any comparison with other types it is an unavoidable conclusion that the inflection of the edge of the blastoderm, so conspicuous in the eggs of the cod and haddock, and other pelagic Teleostean ova, represents in some degree the invagination of the ancestral gastrula. But in the embryos of Elasmobranchs and Amphibians the inflected layer becomes the dorsal

wall of the intestine, or, using the term hypoblast to include all that part of the embryo which is concerned in the formation of the intestine, the inflected layer in those two types is the dorsal hypoblast. It is probable, therefore, that the inflected ring in the Teleostean is the dorsal hypoblast. It has already been pointed out that the whole of the inflected ring, in the process of the envelopment of the yolk by the blastoderm, comes to lie beneath the axis of the embryonic rudiment, between that axis and the yolk. The invaginated layer thus ultimately occupies the same position as the layer in the blastoderm of the bird to which the name hypoblast was first applied. I do not know of any figures of actual sections of pelagic Teleostean ova showing this layer between the embryonic rudiment and the yolk, but it is visible more or less distinctly in transverse optical sections, and such sections are figured by Kingsley and Conn. The final proof that the layer in question is the dorsal hypoblast would be the demonstration that it formed the dorsal wall, and only the dorsal wall of the permanent intestine, the ventral wall being derived from the yolk, *i.e.* from the periblast. This demonstration has never yet been completely effected. Agassiz and Whitman (1) state that they have failed, as did Hoffmann and Ryder, to find any evidence that the periblast forms any portion of the permanent entoderm. The remarks concerning the development of the intestine in the paper of Ryder already quoted (4) convey no information concerning the layers from which it is derived. Balfour 'Comp. Emb.,' vol. ii, p. 61) concludes from some observations of his own that the gut owes its origin partly to nuclei derived from the yolk. It is well known that the intestine in Teleosteans appears first in the greater part of its length as a solid cord; but it seems to me extremely probable that the ventral part of this cord is derived from the periblast. In a former paper (14) on Kupffer's vesicle in the herring embryo, I described some evidence that the intestine in the region of that vesicle is never without a lumen, and that the vesicle is transformed into a portion of the gut by the conversion of the periblastic floor of the former into the ventral

wall of the latter. I have no additional evidence to bring forward; my recent observations have confirmed the view I adopted concerning Kupffer's vesicle, but the formation of the intestine in the region anterior to that vesicle still requires investigation.

It is worthy of remark that the figures and description of the origin of the intestine in the salmon given by Oellacher (11) are not in any way inconsistent with the view for which I am contending, provided it be remembered that the multiplication of nuclei in the periblast probably takes place faster than the consumption of such nuclei in the formation of the floor of the intestine.

The above reasoning leads, then, to the conclusion that the invaginated ring is the dorsal hypoblast, and is therefore homologous with the dorsal hypoblast in other vertebrate types, with the invaginated layer beneath the embryonic rudiment in Elasmobranchs and Amphibians. In the Teleostean there is no wide cavity separating dorsal hypoblast from yolk as in those two types, the only representative of such a cavity is Kupffer's vesicle.

The next question to be considered concerns the method by which the invaginated layer passes, during the growth of the blastoderm, over the yolk from its original position to its final place beneath the embryonic rudiment, and the embryonic ring is absorbed into the dorsal region of the embryo. The first change is part of the second. The main features of the process have already been pointed out, but they must now be examined more closely. In following the process in the living ovum it seems perfectly certain that the centre of the blastoderm (x) at the stage shown in fig. 5 is a fixed point in relation to the yolk, while the non-embryonic portion of the germinal ring slides bodily over the surface of the yolk. There is no doubt a multiplication of cells going on in all parts of the blastoderm during this period of the development as in all others, but this does not account completely for the growth of the embryonic rudiment if its relation to the germinal ring be carefully considered. After the blastoderm has passed the

equator of the yolk the total area of the invaginated layer in the non-embryonic part of the germinal ring is continually decreasing in proportion as the embryo increases. There is no reason to suppose that cells once formed in the blastoderm are again absorbed; it is much more probable that the cells in the invaginated layer are continually multiplying, and the only way of accounting for the layer is that as it slides over the yolk concrescence of the two halves of the ring takes place at the posterior end of the embryonic rudiment; the decrease in the total area of the non-embryonic part of the invaginated layer is thus caused by the continual addition of some of it to the embryonic part. The gradual folding together and concrescence of the diverging limbs of the germinal ring is represented in fig. 26. If this concrescence does not take place after the blastoderm has passed the equator, the cells of the invaginated layer in the non-embryonic part of the ring must either be absorbed, or the invagination process must be reversed and the ring unfolded, both of which suppositions are improbable. The necessity for the process of concrescence is not so evident during the period before the edge of the blastoderm reaches the equator of the ovum, but the process is probably continuous from the beginning of the growth of the blastoderm over the yolk. Fig. 23 is intended to picture the way in which the concrescence takes place, the embryonic rudiment (A B) continually increasing in length as the limbs of the germinal ring are brought together at the point B. No notch like that which is seen at B in this figure is usually visible in the real ovum, but a small indentation was observed in two embryos by Agassiz and Whitman (1, p. 74) in the same position.

The theory that in Teleosteans and Elasmobranchs the embryo was formed by the concrescence of the edge of the blastoderm was propounded by His and Rauber (5, 6, and 7) some years ago. Their arguments in support of it were founded chiefly on the relation of the medullary folds in Elasmobranchs to the diverging limbs of the ring. The theory was opposed by Balfour (' Comp. Emb.,' vol. ii, p. 254),

who considered that the embryo increased in length from the beginning by the addition of fresh somites at the posterior end, as in Chætopods, that is to say, by the multiplication and differentiation of the cells *in sitû* at the posterior end of the embryo, no addition being made to them from other parts of the ovum. As I have pointed out, if Balfour's view were correct it would be difficult to account for the disappearance of the germinal ring in Teleostean embryos. The ring is seen to become part of the embryo, and cannot be accounted for in any other way. Agassiz and Whitman (1) express their adherence to the views of His and Rauber as to the occurrence of concrescence without entering into the question of the significance of the process. Ryder (3) is also fully convinced that the embryo is lengthened by the concrescence along the neural axis of the edge of the blastoderm.

The quotation from His given by Balfour (loc. cit.) is, so far as it goes, completely in accordance with the views in which my own studies have resulted, namely, that in osseous fishes the embryo grows together lengthwise from the two symmetrically-placed halves of the edge of the blastoderm, and that the whole edge of the blastoderm is the blastopore, which thus extends along the whole dorsal median line; but as no re-examination of the subject has been published in this country since the rejection of the whole concrescence theory by Balfour, I have thought it well to show how inevitably the acceptance of the theory follows from a study of Teleostean development in pelagic ova; and I also wish to show how certain of the arguments of His and Rauber concerning the medullary folds in Elasmobranchs are irrelevant, at least to the question of the significance of the concrescence from the point of view of evolution, and to examine into certain consequences which follow from the facts of Teleostean development, consequences which concern the features of development in other vertebrate types, and the relation of these features to ancestral history.

To resume, then: the edge of the blastoderm in Teleostean ova is the primitive blastopore, and the embryo is formed, at all events to a great extent, by the coalescence of the edges of

the blastoderm along the median dorsal line. It is thus easy to see that the formation of the embryo by concrescence in the actual development of the Teleostean is simply the gradual closing of the elongated dorsal blastopore from before backwards. The diagram fig. 27 may be considered as representing the ancestral vertebrate gastrula with its elongated dorsal blastopore, and fig. 28 the same gastrula after the blastopore has closed anteriorly, leaving a small opening at the posterior end of the dorsal surface. The transition from the condition in fig. 27 to that in fig. 28 is represented in actual Teleostean development by the changes shown in the diagrams figs. 24, 25, 26. But we have further to inquire into the relations between the condition of the hypoblast in Teleosteans to that in the ancestral gastrula. It is evident that the part of the periblast on which the edge of the invaginated layer rests at fig. 24, is not the part with which it becomes continuous on the formation of the intestine. In order to understand the Teleostean gastrula we have to perceive that at the period of invagination (fig. 24) a separation takes place between the cells destined to form the sides and dorsal wall of the archenteron and the cells destined to form its floor. In fact the yolk with its periblast represents the part of the hypoblast in fig. 27 between the lines *o* and *p*, and the continuity between the ventral hypoblast and the dorsal is not re-established in the Teleostean until the period of the formation of the intestine.

It is this solution of continuity between ventral hypoblast and dorsal in the Teleostean which enables the invagination to take place all round the edge of the blastoderm at so early a stage. In the Amphibian and Petromyzon no such solution of continuity takes place, and the invagination of the part of the blastoderm corresponding to the point *z* (fig. 24), therefore, does not occur until the yolk is in the position or near it which it occupies at the formation of the intestine. This, it seems to me, is the real explanation of the difference between the invagination in the Teleostean and in the Amphibian. The edge of the blastoderm is, according to what we know at present, finally invaginated round its whole periphery in the frog, and

the posterior end of the blastopore is included in the floor of the medullary canal, remaining open for some time as the neurenteric canal. There is no doubt that the embryo is formed by the concrescence of the lips of the blastopore in the Amphibian as in the Teleostean, but the invaginated ring is not so conspicuous in the former, because the invagination of dorsal hypoblast takes place gradually pari passu with the closing of the blastopore, while in the Teleostean the invagination is completed before the closing of the blastopore begins.

In all this no mention has been made of the formation of the neurochord, because this is a process which is phylogenetically and theoretically altogether independent of the closing of the blastopore. In the Teleostean the thickening of the epiblast which forms the neurochord coincides with the concrescence of the lips of the blastopore, and the thickened column extends down to the edge of the blastoderm, forming the greater part of the thickness of the embryo in the stages shown in figs. 5 to 9. This thickening may begin in the germinal ring before its concrescence, and Ryder states that in Elecate even metameric segmentation of the mesoblast occurs in the germinal ring before it forms part of the embryo; but such facts as these are easily understood on the principal of abbreviation of development, and do not affect the truth that the concrescence of the edge of the blastoderm is simply the closing of the blastopore. The neural cord was originally simply a thickening of epiblast round the elongated blastopore or gastrula mouth, and the formation of the medullary canal began in the course of evolution after the original blastopore had closed up. It is probable that in Teleostei the cells which afterwards line the neural canal attain their internal position during the concrescence, and this supposition is of interest as explaining why no actual inflection from the surface can be made out in the development of the solid neurochord. The formation of the notochord also, in all cases, closely follows the closing of the blastopore.

I would point out here that the primitive streak described in Amphibian embryo, especially in the Triton by Miss Johnson

(10), is simply that part of the blastopore which has just closed; part of it, the extreme anterior part, may be formed during segmentation, but the posterior part must be due to actual concrescence. It will be shown below that the primitive streak in all Vertebrates represents concrescence of the edges of the blastoderm.

The points which are to be emphasised in all this are that the whole edge of the blastoderm in Teleosteans and apparently also in Amphibia is invaginated, and that the whole edge is therefore homologous with the lip of the ancestral blastopore. Unfortunately there is still some uncertainty concerning the relation of the posterior end of the blastopore to the embryo. In Teleosteans, as far as can be seen in the living ovum (*vide* figs. 9 and 22), the posterior end of the blastopore is on the dorsal surface; and it has always been stated that in the frog the end of the blastopore was entirely embraced by the medullary fold and formed the medullary canal. But Sedgwick and Miss Johnson (8 and 9) have lately attempted to show that in Triton part of the blastopore extends round to the ventral surface. Miss Johnson's figures are somewhat diagrammatic. If the proposition is correct that the posterior end of the blastodermic aperture in the newt remains open the question immediately arises, Is the whole edge of the blastoderm in the newt inflected? The answer to which question cannot be found in Miss Johnson's figures. If it is not, then the blastopore (primitive) and the edge of the blastoderm in the newt are not the same thing. If it is, then it may prove true that the anus is derived from the posterior end of the blastopore, and has passed from an original terminal position forwards on the ventral surface by a continuous process during ancestral history without ever closing up. The process may easily have taken place without causing any alteration in other systems of organs except the shortening of the intestine. This would mean that the closure of the medullary canal posteriorly divides the open part of the blastopore into two parts, one forming the neurenteric canal, the other remaining open to the exterior and persisting as the anus.

The yolk blastopore in Petromyzon coincides with the primitive blastopore, but the investigation of this form has not been complete enough to throw much light on the history of the posterior end of the blastopore; the neurochord is at first solid, as in Teleosteans, and, so far as is known, the whole of the blastopore belongs to the dorsal surface.

In Acipenser the edge of the blastoderm is inflected for its whole circumference, and the primitive blastopore is not modified any more than in the types already considered. As far as is known the blastopore at its posterior end is entirely enveloped by the medullary folds. The yolk is contained within the walls of the intestine, but this can easily be explained; the ventral wall of the intestine is formed from the lower part of the nucleated yolk instead of, as in Amphibia, from the upper part. The history of the invagination process in Lepidosteus is unknown.

In all the above types, with the doubtful exception of Amphibia, sooner or later the whole of the edge of the blastoderm is inflected, the yolk blastopore coincides with the ancestral blastopore. In Elasmobranchs this is not so; a great part of the edge of the blastoderm is never inflected. From what is known of the development of this type it seems to me perfectly clear that the dorsal wall of the embryo is formed as in the preceding types by the coalescence of the two halves of the inflected edge of the blastoderm on either side of the embryonic rudiment. But the coalescence of the inflected rim of the blastoderm in Elasmobranchs is not the same thing as the envelopment of the yolk by the blastoderm. A great part of the yolk remains uncovered after the dorsal side of the embryo and the neurenteric canal are completely formed. But it is the uninflected part of the blastodermic edge which coalesces after the period just mentioned. The only way of explaining the relation of the Elasmobranch development to that of Teleosteans and Amphibia, as it seems to me, is the following: —The inflected arc of the blastoderm edge in the former is homologous with the whole of the edge of the blastoderm in the latter, and that arc alone represents the primitive ancestral

blastopore. The rest of the edge of the blastoderm in Elasmobranchs is really a hernia caused by the greater size of the yolk.

The homologous parts in the two cases are represented in the two diagrams I and II, Pl. IV. The uninflected part of the blastoderm edge in the Elasmobranch, c in Diagram II, corresponds with a rupture of the blastoderm in the Teleostean, extending to its edge (x), and represented by the dotted line (c) in Diagram I.

An important question here arises, suggested by the uncertainty before pointed out, concerning the blastopore in Triton. To what part of the embryo does the inflected arc in Elasmobranchs extend? To judge from Balfour's results we should conclude that the neurenteric canal in this type marks the end of the inflection or invagination, and we should be able to decide that the closing of the ancestral blastopore is represented by the coalescence as far as the neurenteric canal and no farther. The results of Sedgwick and Miss Johnson, if correct, must be interpreted in one of two ways. Either the neurenteric canal is in all Vertebrates the termination of the ancestral blastopore, in which case the ventral opening in Triton is simply a hernia corresponding to the yolk hernia in Elasmobranchs, and having no inflection at its edge; or the ancestral blastopore extends beyond the neurenteric canal. If the latter interpretation prove to be true it may perhaps itself be explained thus :—The permanent anus in Vertebrates is derived from the posterior end of the primitive blastopore; on the formation of the neurenteric canal a ventral portion of the blastopore remained open as the anus, and then gradually, remaining functional throughout the process, travelled forwards on the ventral surface. The objection to this view is that such a phylogenetic history ought to have necessitated the existence of a nervous loop anterior to the actual anus, because the primitive blastopore was surrounded by the nerve cord. Even if the hypothesis just stated be correct it is certain that the yolk aperture in Elasmobranchs extends ventrally in front of the actual anus, and therefore must be independent of the ancestral blastopore, must be an embryonic hernia.

It has long been known that the blastoderm in Sauropsida has, in comparison with that of Ichthyopsida, this peculiarity that the embryonic rudiment occupies a central position from the beginning and never extends to the edge. This fact is connected with another, and as will be seen necessarily so connected, namely, that no part of the edge of the blastoderm in Sauropsida is inflected or invaginated to form the dorsal hypoblast. Balfour has given ('Comp. Emb.,' vol ii, p. 238) an explanation of the central position of the embryo in Sauropsida. To quote his own words, "The embryos in Sauropsida have come to occupy a central position in the blastoderm owing to the abbreviation of a process similar to that by which in Elasmobranchii the embryo is removed from the edge of the blastoderm; and the primitive streak represents the linear streak connecting the Elasmobranch embryo with the edge of the blastoderm after it has become removed from its previous peripheral position, as well as the true neurenteric part of the Elasmobranch blastopore." This view is perfectly correct as far as it goes, but the facts need more minute analysis than Balfour undertakes. The primitive streak referred to by Balfour extends from the neurenteric canal, that is, from the posterior extremity of the complete embryo, some distance forward along the ventral median line. But this is not the whole of the primitive streak. There is an anterior part which is contained within the region enveloped by the medullary folds; along the line of this part the epiblast is continuous with the dorsal hypoblast, and actual apertures have been observed through the streak by which the cavity of the commencing intestine communicates with the exterior round the end of the growing notochord. This part of the primitive streak, then, called at its earliest stage the primitive groove, represents the ancestral median dorsal blastopore, and is, therefore, homologous with the whole of the edge of the blastoderm in Teleostei, and with the inflected part of the edge of the blastoderm in Elasmobranchii. The homology is shown in Diagram III, Pl. IV, where y z represents the final position of the neurenteric canal at the posterior end of the complete embryo.

The uncertainty with regard to the termination of the primitive blastopore indicated above occurs again here. If the primitive blastopore is confined entirely to the dorsal surface of the body, its posterior limit is marked by the neurenteric canal, whose position with regard to the blastoderm is indicated by the letter B in Diagram III. In this case the posterior part of the primitive streak in Sauropsida, (c) beyond the neurenteric canal, represents simply the coalescence of the non-inflected part of the edge of the blastoderm in Elasmobranchii is the homologue of the yolk hernia in the latter, and shows that the Sauropsidan embryo in the course of evolution has passed through a stage which is permanent in Elasmobranchs. On this hypothesis, the fact, ascertained by Weldon (10), that in Lacerta the permanent anus is formed at a point in the posterior part of the primitive streak is of little importance; it would simply mean that the line of the first rupture of the blastoderm caused by the increased bulk of the yolk passed through the position of the permanent anus. If, on the contrary, the primitive ancestral blastopore really extends beyond the neurenteric canal, and posterior end of the body, on to the ventral surface, Weldon's results give some support to the view that the permanent anus is derived from the primitive blastopore. In any case it is certain that the yolk in Sauropsida protrudes from a hernia in the ventral wall of the body which is not homologous with the yolk hernia in Elasmobranchs, and apparently not even continuous with that aperture.

The homologous parts in the two cases are indicated by the letter C in Diagrams II and III, Pl. IV. Balfour concludes his remarks on the significance of the peculiar features of the Sauropsidan embryo with the following sentence: "The final enclosure of the yolk in the Sauropsida takes place at the pole of the yolk-sac opposite the embryo, so that the blastopore is formed of three parts—(1) the neurenteric canal, (2) the primitive streak behind this, (3) the blastopore at the pole of the yolk-sac opposite the embryo."

The true state of the case seems to me to be expressed thus :—The real blastopore in Sauropsida, representing the blastopore of the ancestral gastrula, comprises the anterior part of the primitive streak in front of the neurenteric canal, the neurenteric canal itself, and perhaps a portion of the posterior part of the primitive streak. The yolk hernia in Elasmobranchs is represented in Sauropsida by the posterior part of the primitive streak. The edge of the blastoderm in Sauropsida is a new yolk-hernia peculiar to this type, and closing towards the centre instead of from end to end like the hernia in Elasmobranchs. Thus the inflected part of the edge of the blastoderm in Elasmobranchs is homologous with the whole edge in other Ichthyopsida, and no part of the edge of the blastoderm in Sauropsida is homologous with the edge of the blastoderm in Ichthyopsida.

The relations now described are represented graphically by the diagrams figs. 24—30. The diagrams all represent sections through the plane of symmetry of the developing ovum, but the superficial boundary of the blastoderm is represented by the dotted line *e b*, and the internal limit of the dorsal hypoblast in figs. 24, 25, 26, and 29 by the dotted line *s s*. Figs. 27 and 28 are simplified ideal representations of two stages in the development of the ancestral vertebrate gastrula. The stage in fig. 27 corresponds to the stage in the Teleostean represented by fig. 24, while similarly fig. 28 corresponds to fig. 26. The part of the hypoblast in fig 27, between the lines O and P, corresponds to the nucleated yolk (yolk-mass plus superficial layer of periblast) in figs. 24, 25, and 26, and to the nucleated yolk in figs. 29 and 30. The neurochord is purposely omitted in all the figures, because it is only, as it were, accidentally connected with the relations of the blastopore. The notochord (*no.*) and mesoblast (*me.*) are both represented by continuous black shading. The presence of mesoblast between hypoblast and epiblast in figs. 25 and 26 is purely hypothetical; it is probably not developed till after the complete closure of the blastopore. The letter x marks the anterior end of the embryo, the letter y the point at which con-

crescence is taking place, while z marks the posterior border of the primitive blastopore.

The later History of the Periblast in Teleostei.

At later stages of development, after the intestine is completed and the body cavity formed in front of the yolk, the periblast must be considered as belonging to the mesoblast, as, in fact, part of the splanchnopleure. Ryder (3) insists that the chief function of the yolk hypoblast or periblast is the formation of blood-corpuscles, which are budded off from it into the body cavity in Alosa, into the vitelline vessels in Salmo. I have not observed this myself yet, but I do not doubt that it takes place in the herring larva. In the newly-hatched herring there is a space which must b regarded as belonging to the coeloma, situated in front of the yolk, and in this cavity is situated the heart, the hinder end of which is in open communication with the cavity. There is nothing separating the cavity from the nucleated periblast still enclosing the remnant of the yolk, and this fact, together with the formation of the blood-cells from the periblast, compel us to consider the periblast at this stage as part of the splanchnopleure. In many animals, *e. g.* Echinoderms, mesoblast cells are budded off from the hypoblast; in all animals the mesoblast must be originally derived from epiblast and hypoblast, and the conversion of part of periblast in Teleostei into splanchnopleure, without change in its plasmodial constitution, is a phenomenon homologous with the budding of mesoblastic cells from hypoblast in lower forms. It is probable that in other Vertebrates also the splanchnopleure is derived partially from the yolk-cells. Thus the nucleated yolk in Vertebrates contains, in addition to elements destined to form the floor of the intestine, also elements destined to form splanchnopleure and blood. The explanation of the continuity between the body cavity and the cavity of the auricle in Clupea and other Teleosteans has not yet been found, though the fact has long been known. Whether the segmentation cavity is ever actually continued into the

coelomic cavity instead of being obliterated, as Ryder declares to be the case in the cod, I am unable to decide.

Summary.

The ova of Gadus æglefinus, L., G. morrhua, L., and G. merlangus, L., have a perfectly transparent and homogeneous yolk, and a blastoderm of light terra-cotta colour, which disappears at the end of the period of simple segmentation.

The ovum of Trigla gurnardus, L., is larger than those of the three species of Gadus described, and is distinguished by the presence of a large brown-yellow oil globule, which is capable of free motion in the perivitelline space.

The continuity between the cells of the blastoderm and the periblast in its earlier condition can be seen in surface views of the living ova of the cod and haddock.

The invaginated layer of the germinal ring is never continuous beneath the segmentation cavity; it is not continuous with the periblast; it passes to a position beneath the axis of the embryo by the concrescence of the edge of the germinal ring, and the layer constitutes from its first appearance the dorsal hypoblast.

The floor of the intestine is in all probability derived from the periblast.

The whole edge of the blastoderm represents the ancestral blastopore, and the formation of the embryo by concrescence is simply the closing of the blastopore from before backwards.

The edge of the blastoderm in Amphibia, Petromyzon, and Ganoids is homologous with the edge of the blastoderm in Teleostei.

There is some doubt whether the primitive blastopore ends at the posterior end of the embryo, although all that is known concerning the behaviour in Teleosteans favours the conclusion that it does so.

The edge of the blastoderm in Elasmobranchs is not homologous with the edge in Teleostei; the inflected part in the former represents the whole of the latter; the rest of the

former corresponds to a rupture or hernia in the blastoderm of a Teleostean, belonging to the ventral side of the embryo.

The anterior part of the primitive streak in Sauropsida represents the ancestral blastopore. The posterior part represents the coalesced uninflected part of the blastodermic rim in Elasmobranchii. The edge of the blastoderm in Sauropsida corresponds to a hernia in the blastoderm of Elasmobranchs, a hernia which belongs to the ventral side of the body of the embryo.

The periblast in Teleostei after the formation of the intestine forms part of the splanchnopleural mesoblast.

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DESCRIPTION OF PLATES I, II, III, and IV,

Illustrating Mr. J. T. Cunningham's Paper "On the Relations of the Yolk to the Gastrula in Teleosteans and in other Vertebrate Types."

List of more frequent Letters of Reference.

bl. Blastoderm. *e. b.* Edge of blastoderm. *ep.* Epiblast. *hy.* Hypoblast. *g c.* Gastrula cavity. *me.* Mesoblast. *n c.* Neurenteric canal. *no.* Notochord. *pe.* Periblast. *pv.* Perivitelline space. *s c.* Segmentation cavity or blastocœl. *s s.* Internal limit of dorsal hypoblast on surface of yolk, and boundary of segmentation cavity. *v m.* Vitelline membrane. *x.* Centre of blastoderm in its early condition, and anterior end of embryo. *y.* Posterior end of embryonic rudiment, anterior border of blastopore, point at which concrescence occurs. *yk.* Yolk. *z.* Posterior border of primitive blastopore

Figs. 1 to 22 are all drawn from living ova. Figs. 1 to 19 with Zeiss A, Cc. 2, Abbé's camera. Figs. 20 to 22 Zeiss A, Cc. 3, without camera.

PLATE I.

Fig. 1.—Blastoderm of Gadus æglefinus, L., 7½ hours after fertilisation. First segmentation furrow. Optical section in plane at right angles to the furrow.

Fig. 2.—Blastoderm of G. æglefinus, 1 day after fertilisation. Blastoderm forming prominent projection.

Fig. 3.—Blastoderm of G. æglefinus, 1 day 22½ hours. End of period of simple segmentation.

Fig. 4.—Blastoderm of G. æglefinus, 2 days 4 hours. Segmentation cavity formed.

Fig. 5.—Blastoderm of G. æglefinus, 2 days 8½ hours. Invagination of dorsal hypoblast. *i l.* Invaginated layer.

Fig. 6.—Ovum of G. æglefinus, 2 days 7 hours (exposed to higher temperature than ovum from which previous figure was taken). Growth of blastoderm over the yolk.

Fig. 7.—Ovum of G. æglefinus, 2 days 22½ hours. The edge of the blastoderm has passed the equator of the yolk. *o c.* Cerebral part of eye.

Fig. 8.—Ovum of G. æglefinus, 3 days 22 hours. Segmentation cavity not visible in optical section. *an.* Otocyst. *br.* Branchial slits. *i c.* Intestinal cavity. *li.* Liver. *oc.* Eye.

Fig. 9.—Ovum of G. æglefinus, 5 days. Final closure of blastopore.

PLATE II.

Fig. 10.—Ovum of G. æglefinus, 6 days 21 hours. Intestinal tube completely separated from yolk.

Fig. 11.—Blastoderm of G. morrhua, L., 1 day 3 hours. Advanced stage of segmentation.

Fig. 12.—Ovum of G. morrhua, same stage as in last figure, showing continuity of blastoderm with yolk. Origin of periblast.

Fig. 13.—Blastoderm of G. morrhua, 1 day 16½ hours. End of period of simple segmentation. *pe.* Periblast.

Fig. 14.—Blastoderm of G. morrhua, 1 day 21 hours. Segmentation cavity formed.

Fig. 15.—Ovum of G. morrhua, same stage as in last figure. Superficial view of blastoderm.

Fig. 16.—Blastoderm of G. morrhua, 2 days 1 hour. Invagination of dorsal hypoblast.

Fig. 17.—Ovum of G. morrhua, same stage as in last figure. Superficial view of blastoderm.

Fig. 18.—Ovum of G. merlangus, L., 3 days 17 hours. Blastopore nearly closed. *K. v.* Kupffer's vesicle.

PLATE III.

Fig. 19.—Ovum of Trigla gurnardus, 22 hours.

Fig. 20.—Ovum of Clupea harengus, L., 1 day 6 hours. (Temp. about 13° C.) Blastoderm enveloping more than half the yolk.

Fig. 21.—Ovum of Cl. harengus, 1 day 10 hours. Yolk almost enclosed.

Fig. 22.—Ovum of Cl. harengus, 1 day 14½ hours. Closure of the blastopore.

Fig. 23.—Diagram representing pelagic Teleostean ovum in perspective, to illustrate the process of concrescence of the lip of the blastopore.

PLATE IV.

Fig. 24.—Diagram of section of pelagic Teleostean ovum at the completion of simple invagination, before the growth of the blastoderm over the yolk.

Fig. 25.—Diagram of section of same ovum at a later stage, when the blastoderm has covered more than half the yolk.

Fig. 26.—Diagram of section of same ovum, when the yolk is almost covered by the blastoderm.

Fig. 27.—Diagram of the ancestral vertebrate gastrula in section, with elongated dorsal blastopore.

Fig. 28.—Diagram of same gastrula when the closing of the blastopore from before backwards is almost complete.

Fig. 29.—Diagram of section through plane of symmetry of Elasmobranch ovum, at a stage in the growth of the blastoderm over the yolk before the formation of the neurenteric canal.

Fig. 30.—Diagram of section through the plane of symmetry of Sauropsidan ovum, while the neurenteric canal is still open.

Diagrams I, II, III, illustrating the morphology of the blastoderm in different classes of Vertebrates :—I. Condition of the blastoderm in Teleostei. II. In Elasmobranchii. III. In Sauropsida. *x*. Anterior end of embryonic rudiment. *y*. Posterior end of same, at which point concrescence of edges of primitive blastopore occurs. *z*. Posterior end of primitive blastopore. The double line *y z* shows the extent of the rim at which inflection of hypoblast occurs. The single line at *x* indicates the internal limit of the dorsal hypoblast.

On the Structure and Function of the Sphæridia of the Echinoidea.

By

Howard Ayers,
Of Ann Arbor, Michigan, U.S. America.

With Plate V.

Literature.

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A part of the following observations were made some two years ago while studying under Professor W. Faxon at the Museum of Comparative Zoology, Cambridge, Mass.; the other part, with a repetition of the former observations, were made while studying the fauna of the Mediterranean coast of France at the Laboratoire Arago of Banyuls-sur-Mer.

For the opportunity of enjoying the unusual advantages for sea-side study offered by the Arago laboratory I am greatly indebted to its founder and director, Professor H. de Lacaze-Duthiers; and I gladly use this opportunity to publicly acknowledge the great obligation under which I am placed by the liberality with which the facilities of the station were placed at my disposal.

To M. Joubin, in charge of the station, I wish to tender my thanks for many kindnesses shown me during my sojourn at Banyuls.

At the beginning of the last decade Lovén (1) discovered and described certain club-shaped organs occurring among the well-defined group of Echinoderms, the Regularia or Echini, save the single genus Cidaris. None of the other groups of the Echinodermata have as yet been shown to be provided with these organs. The Swedish naturalist applied the very appropriate name of Sphæridia to these organs, and considered them to be sense organs—very probably for the perception of variations in the chemical conditions of the surrounding medium. Lovén has figured and described the various forms of this sense organ from a majority of the genera of the Echinidæ, but does not enter into details of structure, except in the case of the calcareous globule, and here his interpretations are wrong in many points, as I hope to show further on. In the present paper I shall give an account of the structure of the sphæridia in the more important details, and wish to call special attention to the nervous structures of these organs. The observations at the Mus. Comp. Zool. were made chiefly on Strongylocentrotus droebachiensis, O. F. Müll., so common on the New England coast, while at the Laboratoire Arago the sphæridia of the species named below were particularly studied: Echinus melo, Lam., E. esculentus, Linn., E. microtuberculatus, Blainv., Strongylocentrotus lividus, Brdt., S. droebachiensis, O. F. Müll., Sphærechinus granularis, A. Ag., Spatangus purpurens, O. Fr. Müll.

The number and position of the sphæridia on the test is quite constant (except for the very young animal) for the species, and might be used, were it necessary, as a specific character. They are always confined to the ambulacral zones of the actinal surface of the Urchin, and are usually concentrated about the peristome. When they are numerous, as in the Regularia,[1] they may extend in radiating lines to some

[1] Arbacia forms an exception to this general rule, since it has but five sphæridia, one to each ambulacral zone, placed in depressions of the peri-

distance from the peristome; in Strongylocentrotus and Echinus, for example, they extend to the summit of the curve of the test as the Urchin lies abactinal surface downwards. Among these forms they are situated near the edge of the plate nearest the middle of the ambulacral zone, and are seldom more than one to the plate. Among other forms (Spatangus, Clypeaster) they vary greatly in position, and often occur two and three to the plate. In Spatangus and Echinocardium they may be placed in a cup-like depression near the dendritic organs of the peristomial area. Among other genera of the Spatangoid group they are to be found most numerous on the plates of the bivium in a succession of pockets, while on the plates of the trivium they are confined to groups of two or three near the base of the tentacles. The depressions in the calcareous plates mentioned above are changed into closed cavities in some species.

The process consists mainly in the deepening of the cup, but is also accompanied by an overgrowth of the edges of the cup. Such closed cavities are found among the Clypeastroids, Cassidularians, and Spatangoids (Lovénia). In by far the larger number of the species the sphæridia are on the free surface of the test (Regularia and most Spatangoids).

These organs are easily recognisable with the unaided eye, and are about half a milimetre in length, and from one fifth to a quarter of a milimetre thick. They increase in number (but not materially in size) with age, and are renewed like the spines. In the living state the organs are usually colourless, and reflect light with the brilliancy of a diamond, but if exposed to the air for a time they lose their reflecting powers, and acquire a leaden hue, due to the drying of the epithelial layer. The calcareous globule remains unchanged by drying. The movements of the sphæridia consist of rotating and jerking

stomial plates very close to the peristome; among other Regularia they are frequently numerous. I have counted fifty on the test of an adult Strongylocentrotus droebachiensis, and Echinus melo possesses even more. None have as yet been found in Cidaris; and the Clypeastroids and Spatangoids are not abundantly supplied with these sense organs.

motions. The rotating motion resembles that of the spines in every respect; the jerking motion consists of sudden bendings from side to side, and may be observed when the animal is irritated or the organs stimulated, as by the addition of a drop of acetic acid to the seawater in which the Urchin is placed or by raising the saltness of the seawater. If removed from the Urchin in connection with a small part of the test they will remain alive in seawater for several days.

As their name indicates these organs are spheroidal in shape, though varying in form in different species, and not unfrequently in the same individual; thus in Spatangus the normal form seems to be that of an almost perfect sphere, though the elongate form is sometimes found. For Strongylocentrotus droebachiensis the normal form is egg-shape; the spherical form is very seldom seen. There is a typical form for each species, i. e. a particular fashion of the curves of the head and neck common to the majority of the globules of any individual. In each sphærid the following external parts (fig. 20) are readily distinguishable:—The base, which is composed of a mamelon of the test not different from those of the small spines. The joint, that part which forms the connection between the base and globule, and which is composed for the most part of muscle-cells and fibrous tissue in the form of a band surrounding the ball and socket of the joint. The globule or body of the sphærid, which is itself composed of the neck and the head. This method of naming the parts is natural, and is necessary to the proper understanding of the structure of the organ.

From a study of the structure of the calcareous body of the sphærida, Lovén concluded that each globule was composed of two sorts of calcareous matter of different physical properties. That forming the larger part of the body he named the "vitreous calcareous substance," and the branching thread-like network he called the "reticular calcareous tissue." As regards this latter, however, his interpretations are faulty, as the experiments which I will give further on conclusively prove.

The calcareous matter of the globule is a hard and exceedingly brittle, vitreous, transparent carbonate of lime, deposited in smooth or rough (figs. 4 and 19), more or less concentric layers. What appears as calcareous matter in the fresh condition is not entirely such, as the true calcareous substance is deposited in or enclosed between layers of an organic stuff, as to the exact nature of which I am in doubt, though it is probably an albuminoid remnant of the cell walls which enclose the carbonate at the time it was deposited. This animal substance is most conspicuous between two adjoining calcareous lamellæ; it appears in some cases in optical section of the globule in form of radiating threads, which are the bounding lines of elongated cones of the vitreous substance (figs. 2, 5, 7, and 9). In tangential section these cones are circular or polygonal in outline, are largest at the surface, and decrease in size towards the centre of the globule. The radiate cones of adjoining lamellæ (truncate, of course, for each plate save the central one) may not exactly coincide, and there results the appearance shown in fig. 3. This hyaline carbonate does not differ from that composing the spines except in the manner of the arrangement of the parts, and even this difference is only relative since nearly all the stages in the development of the globule from the spine may be observed in the young Urchins (fig. 16 and 17). The structure of the neck of the globule is never very different from that of the spines, while the head is usually so greatly changed by the increase in thickness of the calcareous rods and cross bars and the disappearance of a part of the canals of the spine that its lamellate structure is with difficulty referred to its original type. The elongate forms are frequently rough surfaced, the inequalities consisting of knobs and blunt points, most numerous on the apex of the head, but frequently extending downwards to the neck. They are often perforated by a branch of the canalicular system or may be situated at the side of such an opening.

Canal System.—If the sphæridia, whose canal system it is desired to study, be placed in a small quantity of dilute acid[1]

[1] I used successively H_2SO^4, HNO^3, HCl, chromic, acetic, osmic, and osmo-

under a cover glass one is able to watch the slow removal of the calcareous substance in concentric layers. On such globules, after the acid has acted a short time, the structure called "reticulated calcareous tissue" by Lovén are seen as grooves on the surface of the globule or as knotches at the margin of the same (figs. 9, 18). The canals are often laid bare at one time for more than half the length of the globule. If the sphæridia are treated with caustic soda to remove the animal tissues, the globules washed in water, then in alcohol, heated to drive out all traces of fluids, and then mounted in hard Canada balsam (not a solution in chloroform), which has been warmed sufficiently to allow the globule to sink into the balsam, but not so much that the balsam will enter the open canals, one has a preparation in which the canals instead of appearing as white lines, as they do in the living condition, or as water lines, as they do when simply dried, appear as black rods and lines of various sizes (figs. 5, 6, 7). This appearance is caused by the presence of air in the canals, which owing to the difference in the indices of refraction of the calcareous matter and balsam, on the one hand, and of the air on the other, hinders the rays of light entering the canals from reaching the observer's eye. Again, if globules are treated as above and placed in a solution of safranine or hæmatoxylin after being placed in alcohol, and then removed from the colouring fluid to absolute alcohol and mounted in balsam the canals will be brought out as clear red or purple lines according to the staining fluid used (figs. 4, 10, 11). The colouring matter adheres to the walls of the canals, but does not stain the calcareous matter in the least. Further, if sphæridia are fixed to a slide by means of hard balsam, and their free ends or sides ground off, the canals will appear as grooves on the surface, or as circular or oval openings on the cut surface of the globule.

I think it apparent from these experiments, which were

acetic, osmo-chromic solutions, all of which produce essentially the same effect on the calcareous part of the globule. The acid should not be strong enough to cause an active effervescence.

selected for the reason that they control each other in possible points of error, that the reticulated tissue of Lovén is a system of canals running through the vitreous calcareous substance, and not another sort of finer carbonate. This canal system, as above stated, is but a modified form of the canalicular spaces of the spines. In the neck of the sphæridia the canalicular spaces occupy a large part of the room enclosed by the outer crust, while in the head the meshes are modified into long branching (anastomosing) canals. The central canals, of which there may be few (2—5) or many (10), run perpendicularly through the head and neck. In the neck the structure resembles that of the spines of the species—there being an outer crust and an inner network or reticulation of calcareous bars; both of which possess characteristic forms. In the head the reticulations as such have almost disappeared, persisting only near the centre, their place is filled by long irregular canals, which, however, now and then indicate by the regularity of their arrangement their source (compare figs. 16, 17). In fig. 16 five rows of the external openings of the canal system are shown; in fig. 17 only one such row of canals, leading from the external mouths to the central canals, is shown. The external openings or mouths of the canal system (figs. 1, 4, 5, 6, 7, 8, 10, 15, 16, 17) are usually funnel shape, quite frequently is the inner part of the canal obliterated by the growth of the lamellæ, leaving only the funnel-shaped mouth to indicate its former existence (figs. 4, 7, 8, 10). The internal anastomoses of the canals of the head are very irregular. It is impossible in some cases to trace the connection between the canals of the neck and those of the head (in optical section), the critical point being at the junction of the two parts, *i.e.* at the commencement of the swelling of the globule.

The contents of these canals is mostly nervous cells, though frequently there is found besides the nerve-tissue a chlorophyl-green fluid (*e.g.* Echinus melo).

The soft tissues of the sphæridia are the epithelial covering, the nervous filaments and nerve-cells of the canal system and the contractile and fibrous elements joining the globule to the

test (figs. 12, 15, 20, 26.) In the living condition it is impossible to detect the cell boundaries or nuclei of the epithelial cells, much less those of the more internal nerve-cells, and the presence of cilia is known only by the currents caused by their motion. By adding under the cover-glass a drop of chromic acetic acid solution[1] to the seawater containing the sphæridia the cell-bounding nuclei are rendered distinctly visible and the cilia killed. The latter are long (measuring $1\frac{1}{2}$—2 mm. in length), slender, perfectly homogeneous filaments. They should be studied immediately on the addition of the reagent as they curl close up to the cell wall soon after death (fig. 27). The reagent causes a slight separation of the cells due to the swelling of the cell protoplasm, but otherwise the cells remain in excellent histological condition. The cilia do not regularly occur distributed over the entire surface of the sphærid, as one would infer from Lovén's figure and description; but are usually confined to different sized patches situated on the sides of the neck and globule (fig. 20). The current caused by the cilia varies with the position of the patch. In Sphærechinus the patches on the head set in motion a current toward the base, while those on the neck cause a current towards the head. The epithelium consists of a single layer of relatively large cells resting directly on the calcareous body, underlaid, however, in the neck region by the muscle and fibrous elements of the joint. At the joint it is thickened in the form of a ring (sometimes two, one above and one below the joint), (figs. 1, 15, 20). On isolating the epithelial cells by macerating in osmo-acetic acid solution they are seen to be irregular in shape, having a rounded outer end, a branching inner part, and not unfrequently provided with branches or processes at the sides (figs. 22, 25, *a.b.*) The nuclei are situated in the outer halves of the cells and are frequently surrounded with green chromatophores. In the region of the joint, large, brown or purple chromatophores are of frequent occurrence, they nearly fill the cell in which they are formed.

In studying the nervous tissues of the sphæridia the best

[1] See Fol, 'Lehrbuch d. Vergl. Mikr. Anat., &c.,' pp. 90—100.

results were obtained by the use of chromic-osmic-acetic solution according to Flemming's formula (Fol, loc. cit.). The sphæridia are supplied from the tentacular nerve-trunk, branches of which pass into the globule at the joint. In order to obtain a general view of the nerve-cells of the canal system it is necessary to remove the calcareous matter, and this is satisfactorily accomplished, without injuring the soft tissues, by the use of the acid solution already referred to. It is then easy to trace the nerve-cells from the epithelium to the interior of the globule where they form a network of filaments with here and there irregular knots into which two or more filaments pass. The latter are the nucleated portions of the cells. As represented in figs. 23 and 25, the cells fill partially or completely the canals.

The nervous system of the canals consists of numerous long branching cells provided here and there with ganglionic swellings usually containing a nucleus. These enlargements correspond to lacunæ in the canal system as do the fine filamentous branches to the finer canals (figs. 23, 25.) Such slender filaments are frequently knotted with small granular enlargements exactly such as are found among the nerve-cells of the Medusæ. The branches of the cells anastomose with each other and form thus ring-like meshes in the cell (figs. 25, *c*, IV, V, VI.) These cells reach from the central canals of the globule to the exterior and end in the epithelial layer between the epithelial cells. They pass out of the globule as fine filaments but enlarge at once after passing the orifices of the canal system. Considerable interest attaches to this manner of ending since it reminds one strongly of the end organs of the nerves of higher animals; but is of course not to be compared with them since here we have to do from first to last with a simple cell. The ends are club-shaped or pyramidal with the larger part directed outwards. Whether these nerve-ends are ciliate or not I cannot say, I have never observed anything that would indicate the presence of cilia.

In Spatangus there is usually a quantity of black pigment in the soft tissues of the neck near the base of the head, and

it seems not improbable that in such cases the sphæridia may function as organs of light-perception—*i. e.* serving merely to recognise the presence of light; of course they can have not the least to do with sight.

Theoretical.—The interesting and important fact, made known by the Hertwig brothers in their brilliant study of the nervous system of the Medusæ, that there are frequently developed in this class of animals, standing so low in the organic scale, sense organs of such specialised character that they appear out of all proportion with the degree of development of the nervous system which supplies them, is rendered doubly important when used in comparative anatomy, which discloses to us that among Cœlenterates these organs are frequently as highly developed as analogous organs among worms, molluscs, and tunicates.

Among the Echinodermata we know of eye-spots among the Asteroids (and Echinids?), and of otolith sacs among the Holothurids (Synapta), but these organs never acquire the degree of specialisation seen in the otolith sacs and eye-spots of the Medusæ. From Lovén's description one would not be led to consider the sphæridia of Echinids as so highly specialised as it appears we must now consider them. There is, in truth, a greater specialisation of parts, especially of the nerve-cells among these organs, than is to be seen in similar organs of the Medusæ. Lovén has discussed the probabilities that the function of these structures was either that of hearing, touch, taste, or smell, separately or combined in different ways (*e.g.* as organs with the combined function of taste and smell). The evidence at the present time is decidedly in favour of the view that they possess this double function. The following experiments serve to strengthen this view:—If one adds a drop of dilute acid acetic to the seawater containing the test of the Urchin under observation it is easy to see the sudden stimulation and increased activity of all the external organs—spines, pedicellariæ, sphæridia, &c. The sphæridia are the first to recognise the presence of the acid, and do so by one or two quick, short jerks, followed by a swaying or rotating motion. These motions

are continued until the removal of the acidulated water or until the death of the Urchin. Chromic acid acts much the same as acetic acid. There are two possible explanations of these phenomena: either the sphæridia have for their function the perception of such chemical changes in the surrounding water (*i. e.* taste and smell), and the reporting of the same to the nervous centres of the animal, from whence the intelligence is sent out to the spines and pedicellariæ, which latter are at once alert to secure the food-substance, whatever it may be (*i. e.* in the normal conditions of the animal's life), or the sphæridia are not organs with such function, and are merely more sensitive than the spines and pedicellariæ, which are themselves capable of detecting chemical changes, though in less degree than the spheridia. The first one seems to me the true explanation, especially when the following experiments are taken into consideration.

Sounds, whether loud or low, do not seem to affect the sphæridia in the least; but if the water containing the Urchin be thrown in vibration—as, for example, by striking the glass vessel with some steel instrument a light, sharp blow—the sphæridia are not set in motion, and appeared not to recognise the vibrations; but the spines and pedicellariæ are immediately affected, and begin swaying motions; the spines on that side of the Urchin facing the point of the vessel struck direct themselves towards this point.

EXPLANATION OF PLATE V,

Illustrating Mr. Howard Ayers's Paper, "On the Structure and Function of the Sphæridia of the Echinoidea."

Fig. 1.—A surface view of a living sphæridium of Echinus melo, showing the outer openings of the canal system. The microscope was focussed on the surface of the calcareous body just below the epithelium, which is seen in optical section at the sides of the body.

Fig. 2.—A sphæridium of Strongylocentrotus droebachiensis deprived of its soft tissues by treatment with caustic soda. The concentric lamellæ of the calcareous body appear as layers of unequal thickness in different parts of the globule. The canal system is not figured.

Fig. 3.—The same as Fig. 2, but of another globule. The calcareous body is seen to be made up of three very thick concentric plates, enclosing a central spherical body.

Fig. 4.—A sphæridium of Strongylocentrotus droebachiensis, showing the manner in which the superficial layers of the body grow into projecting tubercles. Caustic soda preparation.

Figs. 5, 6, 7.—Three sphæridia from the same species, treated with caustic soda to remove the soft parts, washed in water and 95 % alcohol, then heated to expel all liquid. The globules were then mounted in hard Canada balsam, warmed sufficiently to allow the bodies to sink into the balsam, but not enough to cause the balsam to enter the canals. The canals are by this means left filled with air, which owing to its different refracting power from that of the calcareous matter and of the balsam, causes the canals to appear as black lines and rods. In Figs. 5 and 6 the balsam has entered the outer branches of the canal system, and in consequence the canals here can be distinguished merely by the line of contact between the balsam and calcareous substance.

Fig. 8.—A part of the canal system of a sphæridium of Strongylocentrotus droebachiensis, projected on the plane of the paper. Caustic soda preparation.

Fig. 9.—A sphæridium of the same species, treated with diluted HNO^3. The calcareous matter has been partly dissolved, thus exposing the internal canals as grooves. At the edges of the body they appear as notches in the body.

Fig. 10.—A sphæridium of Strongylocentrotus droebachiensis, which has been treated with caustic soda, washed in water and 95 % alcohol, placed in an aqueous solution of safranine, passed through absolute alcohol and clove oil and mounted in balsam. The canals were filled with the stain-

ing fluid, which now adheres as a dry film to the sides of the canals. The calcareous substance is not in the least coloured.

Fig. 11.—From the same preparation as Fig. 10. The canals are arranged fan shape (in optical section), and are to be traced from the reticulate canal system of the neck to the surface of the globule, on which most of them open.

Fig. 12.—Cells from a sphæridium of Echinus melo. *a.* A large cell, with chromatophores and vacuole from beneath the epithelium of the base of the organ. *b.* Surface view of five epithelial cells. From an osmo-acetic-carmine preparation.

Fig. 13.—An optical section of a portion of the epithelial cover of a sphæridium of Echinus esculentus. At xxx are shown the ends of nerve-cells, which pass out to the surface of the organ through the canals in the calcareous substance (here dissolved away) and between the epithelial cells. They end at the surface in knot-like enlargements. Chromic-osmic-acetic solution, stain Ranv. pic. car.

Fig. 14.—An optical section and a surface view of the epithelium of Echinus melo. *a.* Optical section from the apex of the organ, showing the numerous thin layers of slime or mucus surrounding the globule in the living state, and through which the cilia pass. *b.* Surface view: in three of the cells are shown the chromatophores, which are of common occurrence.

Fig. 15.—A fresh specimen of a sphæridium of Strongylocentrotus droebachiensis. The upper end of the calcareous globule is broken off, and the epithelial layer is ruptured. The nervous system is seen to extend into and fill part of the canals.

Fig. 16.—A sphæridium from Spatangus purpureus, treated with caustic soda. Surface view, showing the openings of the canal system.

Fig. 17.—The same as Fig. 16, seen in optical section. The figure shows three of the principal canals, the canalicular network of the neck (neither of which open on the surface in this globule), and also the lateral branches of the left central canal; the side cells establish communication with the epithelial layer.

Fig. 17, *a.*—*x.* A portion of the left stem shown in Fig. 17, and one of its side branches, with its outer opening. *z.* A portion of the canalicular network of the neck further magnified. The shaded parts represent the canals.

Fig. 18.—A living sphæridium of Spatangus purpureus, treated with dilute acetic acid until the superficial layers of the calcareous body are partly dissolved away. The epithelial cover is ruptured to expose the globule. A part of the canals are left as grooves in the new surface, appearing as notches at the margin of the globule.

Fig. 19.—An optical section of a sphæridium of Echinus melo, showing the structure of the calcareous part. This globule is composed of wavy concentric lamellæ and radiating cones.

Fig. 20.—A sphæridium of Echinus esculentus, to illustrate the distribution of the cilia, chromatophores, and the relation of the parts of the organ. *a*. The basal articulation (a knob of the test). *b*. The joint, composed of muscle-cells and fibrous bands. *l*. The limiting line between the epithelium and tissues of the joint. *c*. The neck. *g*. The red and brown chromatophores most plentiful in the region of neck and base. *d*. The head of the sphæridium. *e*. The epithelial covering. *f*. The thickened ring of epithelial cells surrounding the neck and the base. *g'*. A large cell, such as is found frequently in the base. *h*. The green chromatophores (chlorophyll?). *i*. Finger-shaped processes of the base. The base also frequently bears one or two small pedicellaria. *w w*. Patches of vibratile cilia.

Fig. 21.—Muscle-cells from the joint of a sphæridium of Strongylocentrotus lividus. From an organ macerated in osmo-acetic solution (Hertwig's), stained in carmine mounted in glycerine.

Fig. 22.—Epithelial cells from Echinus melo. Chromic-acetic maceration preparation. Ranvier's carmine, glycerine.

Fig. 23.—The central part of a transverse section of a sphæridium of Echinus esculentus, through the neck, showing the remains of the calcareous plates (*a*), and the nervous matter of the canals (*b*).

Fig. 24.—A surface view of the epithelial layer of a sphæridium of Echinus melo, showing chromatophores.

Fig. 25.—Muscular and connective-tissue cells (*a*, *b*, *b'*), and nerve-cells (*c*), from a sphæridium of Echinus melo, treated with chromo-osmo-acetic (Flemmings's) solution; stained in carmine, mounted in glycerine.

Fig. 26.—A transverse section of the sphæridium of Echinus melo, through the head of the organ, after treatment with chromo-acetic, carmine, clove oil, paraffine.

Fig. 27.—Three stages in the death of the cilia of Sphærechinus brevispinosus. *a*. The slowly-vibrating cilia immediately after the action of the chromo-osmo-acetic solution. *b*. The resting cilia just after the cessation of motion. *c*. Five minutes after death. A little later the cilia have curled close up to the epithelial surface.

The Nerve Terminations in the Cutaneous Epithelium of the Tadpole.

By

A. B. Macallum, B.A.,
Fellow of University College, Toronto, Canada.

With Plate VI.

I.—Historical.

The earliest recorded observations on the termination of nerves in the skin of the tadpole were made by Hensen[1] in 1864. According to this observer a plexus of nerve-fibrils, situated immediately beneath the homogeneous membrane or larval corium, gives origin to fine fibrils, which pass through the membrane and terminate each in the nucleus of an epithelial cell.

A little later Eberth[2] found the coarser nerve-fibres of the tail to end in a network of anastomising fibrils, situated somewhat deeper than the plexus of Hensen. He was unable to trace any fibrils beyond this network although he observed them in the corium of the adult frog.

In connection with these observations Eberth described structures of a peculiar nature which he discovered in cells of the epithelium of the tadpole. As these structures are of special interest, bearing on the subject of the present work, a brief abstract of Eberth's description of them is here given.

[1] 'Virchow's Archiv,' Bd. xxxi, p. 51; also, 'Arch. für Mikr. Anat.,' Bd. iv, p. 111.

[2] 'Arch. für Mikr. Anat.,' 1866, Bd. ii, p. 490.

They are present during larval life only, and appear first in tadpoles of 3½ cm. length. The refracting, colloid substance of which they are formed is not easily attacked by reagents, but various colouring matters stain it quickly. They are apparently wholly absent from the cells of the superficial layer of the epithelium, and when occurring in those of the basal layer they rest with an expanded foot on the corium. They are easily isolated from the cells containing them, and if the epithelium is brushed away, many of them are found to be attached to the corium. Their shape varies greatly, being sometimes fusiform, sometimes rodlike or fibrous, and at other times resembling closed, or open rings, or spherical masses. Some of the elongated or rodlike forms appeared to be constituted of a central axis and a more refracting sheath.

Eberth at first considered these structures to be intracellular nerve terminations, but on subjecting them to the action of gold chloride could find no nerve-fibrils connected with them. As to their significance he could give no definite opinion other than that they are secretions of the protoplasm which arise first in the immediate neighbourhood of the nucleus.

Leydig[1] has recently described these structures, now termed the figures of Eberth, as they occur in the skin of the larvæ of Hyla arborea and Pelobates fuscus, and has put forward the view that they are comparable to the fusiform bodies in the cutaneous gland cells of certain Gasteropods or to the urticating threads in Cœlenterates.

To return to nerve terminations proper. Klein[2] has given a description of the distribution of peripheral nerve-fibres in the tail of the tadpole of Hyla. He found a plexus of non-medullated fibres situated below the corium, and giving rise to finer fibres, which, approaching the epithelium, anastomose

[1] "Neue Beiträge zur anatomischen Kenntniss der Hautdecke und Hautsinnesorgane der Fische." ('Sonderabdruck aus der Festschrift der Naturforschenden Gesellschaft zu Halle,' Halle, 1879.)

[2] 'Handbook for the Physiological Laboratory,' 1873, p. 80. I have not, unfortunately, access at present to the important work by the same author, of which the paragraph in the Handbook is evidently but a short abstract.

with each other to form a second plexus. The finest fibrils are described as occurring immediately underneath the epithelium in a network, the meshes of which are so narrow that several of them can be covered by the nucleus of an epithelial cell. As no nerve-fibrils could be traced beyond this network, Klein concluded that they terminate in it.

Leboucq[1] also found a terminal sub-epithelial network in the skin of the larvæ of Pelobates and Triton, its meshes, however, not being so narrow as those of the network described by Klein. He observed, also, nerve-fibrils terminating in homogeneous finely-granuled corpuscles situated among the epithelial cells, and provided with processes which end in the intercellular substance. Leboucq compared these corpuscles to the cells of Langerhans in the Malpighian layer of the human skin, which acquire a violet tint when treated with gold chloride, and which are considered by some observers to be the end-organs of intra-epithelial nerve-fibrils. He believed that some of the fibres terminate in the nuclei of the cells described by Leydig under the name of "Schleimzellen."

Pfitzner's work,[2] next in the order of time, deals specially with the intra-epithelial terminations of nerves. This observer, by first hardening the tissue with chromic acid, then treating the separate sections with gold chloride, found that the figures of Eberth attained in every case a violet tint, and he considered them consequently to be nerve terminations. He also, by the employment of saffranine, as well as by the use of gold chloride, observed that these structures are continued through the corium into the subcutaneous tissue. According to Pfitzner every epithelial cell has two figures of Eberth in its interior, which terminate in knob-like swellings near to, but never touching, the nucleus.

Now, in the plate accompanying Pfitzner's work there is apparently no resemblance between the figures of Eberth, as one usually sees them, and the nerve endings there represented. Canini,[3] who followed Pfitzner's methods of research,

[1] 'Bulletins de l'Academie Royale de Belgique,' 1876, p. 561.

[2] 'Morph. Jahrbuch,' Bd. vii, p. 726.

[3] 'Arch. für Anat. und Phys.,' Phys. Abth., 1883, p. 149.

has pointed out this fact, and although he observed the passage of fibrils through the corium and their connection with the figures of Eberth, yet he was unable to form any opinion of the significance of either.

Mitrophanow,[1] by employing the ordinary method of using gold chloride, and comparing its results with those obtained by Pfitzner's method, came to the conclusion that in each of the two methods gold chloride selects entirely different tissue elements; that in the first nerve structures are stained deeply while the figures of Eberth remain uncoloured, and in the second method the chromic acid changes the chemical relations of the figures of Eberth in such a way that the latter become capable of impregnation with gold. He pointed out also that these structures are never so regular as Pfitzner has represented them, and that they are in no way connected with the fibrils which pass vertically through the corium, and which, in his opinion, are of connective-tissue origin. He found the coarser nerve-fibres of the skin arranged, as Hensen and Eberth described, in a plexus under the corium, and giving origin to fibrils which pass through that membrane to terminate between, but never within, the cells of the epithelium. With Pfitzner's methods he was unable to find any intercellular terminations.

II.—Material.

My researches were carried on with the tadpoles of Rana halecina. These measured from two to two and a half inches in length, and several of them presented quite distinctly the outlines of the posterior limbs. In order to get characteristic preparations of the epithelium I found it necessary to keep the tadpoles in perfectly normal conditions, *i.e.* in water regularly renewed, and of a constant temperature. With the water unchanged for several days, or with its temperature considerably lowered for an equal length of time, the number of layers in the epithelium was found reduced to two, which apparently soon increased to three, four, and even five if the tadpole was again subjected to normal conditions. This reduction in the

[1] 'Arch. für Anat. und Phys.,' Phys. Abth., 1884, p. 191.

number of layers was accompanied by other changes, which will be referred to farther on. I think that these points are important, for some of the results of Pfitzner's researches might be accounted for on the ground that he used material which was in such abnormal conditions.

III.—Methods of Study.

For the purpose of hardening the epithelium for vertical sections Erlicki's fluid and solutions of chromic acid of different strengths were employed. The former reagent is not suitable for anything else than preserving well the outlines of the cells and for the figures of Eberth, to which it gives a full plump appearance. It is of no value for karyokinetic figures, which, after its use, have the appearance of a scattered granulation, and it renders the intercellular bridges invisible. For preparing these, as Pfitzner recommends, chromic acid is the best reagent, especially when used of the strengths of one sixth and one third of 1 per cent. For the figures of Eberth, however, it has not in my hands proved as suitable as Erlicki's fluid.

For staining the figures of Eberth nigrosine is to be specially recommended, because it gives to them a deep dark-blue colour, while the epithelial cells and their nuclei take but a very slight shade. In this way most of the finer processes of a figure can be followed throughout a cell. Saffranine is also to be recommended, as it gives a deep stain to the figures, but it has one disadvantage compared with nigrosine, in that it is difficult to get a successful preparation with it without at the same time obtaining the cell protoplasm more or less diffusely stained.

In order to obtain sections stained with both nigrosine and saffranine the following method was adopted:—The tail of the specimen, hardened in Erlicki's fluid, and the reagent extracted with weak, then with strong alcohol, is put for thirty or forty hours in a solution of nigrosine, made by dissolving 0·1 grm. of the latter in 4 cc. of distilled water and adding this to 96 cc. of strong alcohol. The excess of the nigrosine is extracted with alcohol, and from the tissue embedded in paraffin vertical

sections, of 8—10 mm. in thickness, are cut, and then by Schällibaum's method fixed on the slide. After removing the paraffin, and washing the slide for some time in absolute alcohol, it is put for two or three minutes in a solution of saffranine of the strength recommended by Pfitzner: 1 grm. saffranine, 100 cc. alcohol, and 200 cc. distilled water. The slide is afterwards washed in distilled water to remove the excess of the colouring matter, and then put in absolute alcohol. The time during which the slide with the sections on it must lie in alcohol must be determined by experience, for if left one minute too long the whole of the saffranine is removed. The sections are cleared up in turpentine, and mounted in dammar or balsam.

In spite of the uncertainty of this method of double staining there is always in each section, if it has not been allowed to lie too long in alcohol, a number of places where neither too much nor too little of the saffranine has been extracted, and where one obtains, consequently, such relative effects of the two stains as is indicated in figs. 1, 2.

Of the various methods of employing gold chloride to demonstrate nerve structures in epithelium I have, after a careful comparison of the results obtained by each, adopted and followed one for the greater number of my experiments. I did not deem it of any value to try the method employed by Pfitzner, seeing that it is open to the objection urged by Mitrophanow against it, that chromic acid prepares non-nervous structures for impregnation with gold. Canini found the same method to give no results at all for the epithelium of higher Vertebrates. The method which I followed, and which was employed by Mitrophanow also, consists simply in treating the perfectly fresh tissue with a 1 per cent. solution of gold chloride for an hour, then washing it in distilled water, and finally placing it in a solution of formic acid, made in the proportion of one part of the acid to ten of distilled water. Kept for about thirty hours in this fluid with complete exclusion of light the tissue will have at the end of that time a deep violet colour. To complete the success of the

preparation it is placed in a mixture of equal parts of glycerine and water, with a drop or two of formic acid for every 10 cc. of the mixture. It is much improved by keeping in this fluid a month or more. The same fluid should be used in mounting pieces of the preparation.

By a careful attention to the details of this method I succeeded in obtaining in the greater number of cases very successful preparations. Acidification of the tissue with lemon juice or formic acid previous to treatment with gold chloride did not enhance the success of the results, while it often appeared to have a contrary effect.

IV.—The Skin.

The height of the epithelial layer on the tail varies considerably in different parts of a vertical section. Near the middle of the lateral surface it is very often one and a half times what it is along the border, and between these points there are gradations in thickness. It sometimes happens that the number of layers in the epithelium may be greater near the middle of the lateral surface than elsewhere. The thickness of the epithelium varies also with the age of the tadpole.

The number of layers in the epithelium of course influences its thickness to a certain extent. If the tadpole has been kept in favorable or normal conditions, the number of layers which can be easily distinguished is then three, often four, or five. Of these, the two most distinct from each other are the superficial and basal layers; while the others show stages intermediate between these two. The constituents of the basal layer are the largest and are cylindrical in shape. The nucleus is usually placed in the upper half of the cell when there are only two layers in the epithelium, but in the lower half of the cell when there are more. The cellular contents possess few or no granules, and apart from the figures of Eberth are clear and transparent. The cells of the intermediate layers resemble those of the basal layer in this respect. These are usually of a polyhedral shape.

The superficial layer is composed of cells cubical or flattened with the outer, and often the lateral walls, remarkably thickened. Indeed, this thickening is not confined to the superficial cells but also occurs in those immediately under them (fig. 2, *d*). It seems to be due to a horny deposit brought about by degradation of the cellular contents, and it sometimes takes a granular form which disappears after a treatment with acids.

Between the basal cells and between most of the cells of the intermediate layers, one can distinguish, in chromic acid preparations, the intercellular bridges. These have completely disappeared from the superficial cells. Very often on treatment with gold chloride the fluid circulating between the bridges in the intercellular passages is coagulated in the form of minute bluish droplets. These droplets are lost when the cells are isolated.

The corium on which the epithelium directly rests is a thin membrane formed of a fibro-gelatinous substance, the arrangement of the fibrillæ, when they can be determined, being parallel to the general surface. There are no cellular or nuclear elements which pertain properly to it, although corpuscles of connective tissue situated below the membrane sometimes appear to be closely connected with it. Such cellular elements as are usually seen in the membrane are amœboid corpuscles on their way to or from the epithelium. At the time that resorption of the tail commences the fibrillæ of the membrane tend to separate widely and give then all the appearances of the adult corium.

V.—The Nerve Terminations.

The figures of Eberth are to be found in all the layers of the epithelium, although only exceptionally in the superficial one. The reagent which serves to show them best is nigrosine which gives them an intensely dark-blue colour. When a section is thus stained, one can sometimes see the figures as minute beads in the superficial cells. Those of largest size and oddest shape are to be found in the basal cells, but they

are more developed and more numerous when the epithelium is four or five-layered. This is contrary to what Mitrophanow observed, who contends that they are only to be found in the basal cells, from many of which they may be absent, and that they are most prominent when the epithelium consists of two layers only. My observations cannot confirm either of these statements. A figure of Eberth is never absent from a basal cell and is to be found in the great majority of those of the intermediate layers.

I need not describe the figures of Eberth more fully, since I have given at the commencement of this paper the substance of Eberth's own description. For the appearance presented by these structures I refer the reader to figs. 1 and 2, *e*.

If sections stained with nigrosine be treated with a saffranine solution in the manner already indicated, it will be seen in a large number of cases that one or more red fibrils run in the axis of a figure of Eberth, which retains its deep stain. If a figure coils around a nucleus, a red fibril will be found to traverse the course of the coil. Most of these fibrils terminate in minute knoblike swellings within the body of the figure itself, or in one of its finer divisions.

All red fibrils in these sections are not found inside figures of Eberth. Between some of the basal cells one can very often see such a fibril passing upwards from the corium to terminate in a figure of one of the cells of the intermediate layers. Again, when a figure of Eberth terminates near the lateral or upper wall of a cell its fibril may pierce the cell wall, enter the intercellular spaces, and, after a certain distance, penetrate the figure of a cell of an intermediate layer. It may divide before its termination, or after it has penetrated the figure. An example of the latter occurrence is seen in fig. 2. The distribution is, however, very irregular. One or more of the branches of these intercellular fibrils may terminate in figures of Eberth in cells of the intermediate layers, while others may end in minute beads which lie free between the cells. These fibrils as a rule do not pass directly through the corium into the subcutaneous tissue. The majority of those which

come through the corium directly do not branch, are somewhat thicker than the others, and have a larger intercellular bead-like swelling. These never terminate within the cells and are not numerous. All the other fibrils seem to start at the upper surface of the corium. I have drawn an exceptional case in fig. 1, *n''*, where a fibril was seen to pass directly through the corium and into the axis of a figure of Eberth. One can very often see a series of fibrils, at regular distances from each other, pass through the corium and terminate at the line between it and the epithelium. Sometimes their terminations are under the expanded foot of a figure of Eberth which then appears to be connected with them. Canini[1] has drawn a case of this apparent connection.

It must be observed, once again, that the method of staining these fibrils is not always successful. Nigrosine stains the figures of Eberth, while saffranine attacks the fibrils. If, however, the section to be stained be left in the saffranine solution too long, the figures take up the colour and have now a dull red tint. On the other hand, there is always great difficulty in preventing all the saffranine from being extracted with absolute alcohol. There is another disadvantage frequently resulting from the use of this reagent, that the epithelium throughout a section does not acquire an equal depth of stain. This irregularity, which is referred to by Pfitzner, is, I think, not due to any faulty method of manipulation.

The fibrils which are stained red with saffranine are nerve-fibrils. To prove that they are such it is only necessary to treat the epithelium with gold chloride in the manner already described. If a thin piece of the tail, prepared in this way successfully, be mounted with its epithelium intact, and the tube of the microscope be so placed that the superficial layer is in focus, such a view is obtained of its cells as is represented in fig. 3. As a rule, the cells appear as there indicated. At other times, however, they seem to be made up of polygonal fields of bluish-tinted granules, in which the outlines of the nuclei are not often visible. Between neighbouring cells can

[1] Op. cit.

be seen minute beadlike bodies of an intensely violet colour, which, if the micrometer screw is moved gently and slowly, can be observed to pass below into minute fibrils of the same colour. These are the intercellular nerve terminations described by Mitrophanow, who, however, does not represent them in his figures as numerous as they really are.

But this is not all that can be seen in the same field. If one carefully scans the optical section of one of the cells, points much smaller than the intercellular ones appear in the immediate neighbourhood of the nucleus. Careful focussing also shows that the majority of them are simply the terminations of minute intracellular fibrils. Very often these points rest above the nucleus, and the fibrils connected with them take a curve corresponding to the surface of the nucleus, bending around and under it. Two, three, and sometimes four and five such fibrils can be found in a cell. They are undoubtedly nerve terminations, for their origin can without difficulty be found in the intercellular fibrils. Fig. 3 gives a view of the relative sizes of the two modes of termination. Both in successful gold preparations have the same depth of tint.

If now it is desirable to see more definitely whether the fibrils which appear within the cell are really within, portions of the epithelium must be taken which have been some hours longer in the reducing fluid than is usual. A number of cells will then be completely isolated, and one can see distinctly the intracellular fibrils and endings in each, sometimes a little above, sometimes in a plane with the optical section of the nucleus. Fig. 4, *a* represents a view of one of the isolated cells. In these same preparations it is sometimes possible to see the intercellular endings as club-shaped, deep violet bodies lying scattered in the mounting fluid.

Sometimes in a superficial cell a series of granules, brownish, but less intense in colour than pigmentary substance, is arranged in the form of a curve around the nucleus, in such a way as to give rise to the idea that they are the degradation products of a figure of Eberth.

In focussing for the cells of the intermediate and basal

layers of the epithelium, in the same preparation intercullular and intracellular fibrils and terminations can be seen as easily as in the superficial layer. Here also the intercellular terminations are much larger and more distinct. If the preparation is one in which the intercellular fluid is precipitated in the form of bluish droplets, there is no necessity for isolation of a cell in order to determine whether nerve-fibrils terminate in its interior, because all the outlines of the cells are rendered very plain. A study of the optical section of one of these cells gives the same results as in the case of one of the superficial cells. In fig. 5 several of the cells of the basal layer are represented with their nerve terminations.

In some preparations the figures of Eberth cannot be seen at all owing probably to some unfavorable action of the reducing fluid. Where, however, they appear quite distinct one or more violet fibrils are seen to terminate in them. The figures remain colourless in the more or less tinted protoplasm of the cell. It is not uncommon when the preparation has stood long in formic acid to have a number of the cells broken down, with the figures lying free in the mounting fluid. Then one can see very plainly in them the axial, violet-coloured fibrils and their beadlike terminations as indicated in fig. 7.

This reveals plainly that the figures of Eberth are simply sheaths for intracellular nerve terminations. As such they exist all over the body of the tadpole, and are not confined to the tail. Fig. 6 is drawn from one of my preparations of the skin in the immediate neighbourhood of the mouth. In this case the branching nerve-fibril with three cells lies isolated from the rest of the tissues in the mounting fluid. There one of the branches of the fibril is seen to terminate in the interior of an oval refracting body, a figure of Eberth, from which the cell enclosing it has been torn away.

These figures of Eberth have been compared to the clavate cells in the skin of Petromyzon and Myxine. But the structures known as the cells of Leydig in the skin of Amphibian larvæ are really clavate cells, and in these I have sometimes seen figures of Eberth. The cells of Leydig are not very

numerous in my preparations, and I had but a few opportunities for observing them well. From such as were examined, however, I find their terminal intracellular nerve-fibrils are more numerous than is the case with the other epithelial cells.

How came Pfitzner to mistake the figures of Eberth for nerve terminations?

From my own observations of these structures in various stages of larval life, and from a comparison of these observations with figs. 1 and 3 of Pfitzner's work, I am led to believe that he took for typical the forms as they are found a short time before the commencement of the resorption of the tail. At this time the majority of the figures of Eberth are slimmer, and invest the nerve-fibrils closely. By comparing his fig. 3 with fig. 3 given here it will be seen that the nerve terminations indicated by him are similar in every respect to those drawn by me, save that he recognises no intercellular endings. His method of manipulation accounts for the supposed nerve terminations being so large, as the figures of Eberth are stained, and not the fibrils occupying their axes. Pfitzner is wrong also when he describes the intercellular fibrils as having, each of the two, a different origin. As I have already stated the fibrils supplying the different cells take their origin from a set of fibrils on a level with the superior face of the corium.

Regarding the arrangement of the coarser subcutaneous nerve-fibres in what is now termed the fundamental plexus, the observations of Hensen, Eberth, Klein, Leboucq, and Gaule[1] practically agree, and their descriptions are so complete that I am unable to add anything of importance. The second plexus described by Klein I have not seen, and I am convinced from a study of vertical sections that the fibrils which pierce the corium arise, without the intermediation of a second plexus, direct from the fundamental plexus. Gaule's secondary plexus, if I understand his description rightly, is placed below the corium, where I cannot find the slightest traces of it. It would, if placed above the corium, seem to agree with the

[1] A continuation of Canini's work, 'Arch. für Anat. und Phys.,' Phys. Abth., 1883, p. 154.

terminal sub-epithelial network of Klein and Leboucq. This network, as I find it, rests directly on the corium, and its meshes are very often as narrow as those described by Klein. The excessively fine fibrils forming them anastomose with each other, and at points along their course can be seen large numbers of delicate swellings, which serve as points of origin for intra-epithelial nerve-fibrils. These may terminate directly either within or between the cells, or they may branch more than once, the delicate twigs resulting in this way terminating differently also.

All intra-epithelial nerve-fibres do not originate from this sub-epithelial network. In preparations where the gold has not coloured the epithelium too strongly, one can see in the fundamental plexus a certain number of fibres, each with a series of regularly-placed swellings which give origin to single fibrils passing through the corium and ending without branching between the cells of either the basal or first intermediate layer of the epithelium. These fibrils and their comparatively large, swollen, beadlike terminations, can be easily seen with a low-power objective, such as Hartnack's No. 4, and have been already referred to by me, when describing sections prepared with nigrosine and saffranine. The terminal beads are from three to five times the size of one of the intercellular terminations of fibrils of the sub-epithelial network. They are best shown in preparations were the gold treatment has been only partially successful and where the other terminations are not seen.

Mitrophanow has evidently seen these as well as the ordinary intercellular nerve terminations, and he confuses the two kinds. In the woodcut accompanying his work, he indicates the terminal fibrils as passing directly from the fundamental plexus through the corium and terminating in large beads between the epithelial cells. In his figs. 1 and 2, he represents the same kind of fibrils but he has not given them terminations as large as I find them to have. He observed no sub-epithelial network, but from his fig. 3 he appears to have seen the intercellular terminations of fibrils arising out of it.

I am, therefore, inclined to believe that Mitrophanow drew his conclusions from a study of preparations not sufficiently successful. In no other way can I account for his confusing two kinds of terminal nerve-fibrils and for his overlooking the sub-epithelial network. If this explanation is correct, then it will be easy to understand why he observed no intracellular nerve terminations.

It seems remarkable that the intra-epithelial nerve-fibrils should take their origin, some from the fundamental plexus, some from the sub-epithelial network. There must be some physiological significance in this arrangement. Pfitzner believes that each of the two fibrils described by him as terminating within every epithelial cell represents a channel for a special kind of nervous impulses, one fibril corresponding to a path for motor, the other to a path for secretory impulses. The intracellular fibrils, as I have found them, arise from the sub-epithelial network, and the number distributed to each cell is not limited to two, but is very often more. Consequently, one can hardly imagine that they have different functions to perform. On the other hand, the different modes of origin of all intra-epithelial fibrils, as described above, might well be supposed to correspond to some differences in function. In that case the fibrils from the fundamental plexus terminating directly between the epithelial cells might serve as paths for sensory impulses, while the sub-epithelial network with the intracellular and intercellular fibrils arising from it might conduct secretory or trophic impulses.

I am unable to suggest any reason for the occurrence of more than one termination within every cell.

The question may be asked, why the intracellular fibrils of the basal and intermediate layers of the epithelium possess sheaths in the form of figures of Eberth, while those of the majority of the superficial cells have no such sheaths? Also, why are the figures of Eberth completely absent from the skin of the adult frog? These questions are difficult ones to answer, but I may suggest several data which will, probably, assist in their solution. The cells of the intermediate and

basal layers of the epithelium undergo vital processes much greater than those of the superficial layer or than those of the epithelium of the adult. Again, the figures of Eberth are most highly developed when the epithelium is constituted of from three to five layers of cells, and they almost wholly disappear when the vital energies of the cells containing them are spent, as, for example, at the commencement of resorption of the tail. Do these facts point to the supposition that the figures of Eberth protect the intracellular nerve-fibrils from the vital processes, assimilatory or otherwise, of the vigorous cell?

Nussbaum[1] found in the cells of the pancreas of Salamandra maculosa structures which have been termed by him "Nebenkerne," and which are apparently similar in many respects to figures of Eberth. They are rarely visible when the cell has undergone a somewhat prolonged period of rest, and they attain their most marked development four or five days after the animal has taken food. They have often many of the curious shapes assumed by the figures of Eberth, and one or more may be found in a cell, situated between the nucleus and the membrana propria.

It is quite probable that these also are sheaths for intracellular nerve terminations.

VI.—Summary.

The results of this work may be summarised in the following statements:

1. Certain fibres of the nerve network, situated below the corium, and known as the fundamental plexus, give origin to fibrils which enter the epithelium and terminate in comparatively large beadlike bodies between the cells.

2. From a network of fine anastomising nerve-fibrils situated immediately below the epithelium, and forming meshes, each narrower than the surface covered by an epithelial cell, arise other excessively fine fibrils, which end either within or between the cells, or, after branching, in both fashions.

[1] 'Arch. für Mikr. Anat.,' Bd. xxi, p. 337.

3. One, commonly two, often three or more, nerve-fibrils terminate in the interior of each epithelial cell near its nucleus.

4. The figures of Eberth are sheaths for intra-cellular nerve terminations.

EXPLANATION OF PLATE VI.

Illustrating Mr. A. B. Macallum's Paper on "The Nerve Terminations in the Cutaneous Epithelium of the Tadpole."

In drawing Figs. 1 and 2, Hartnack, obj. 7, and Oberhäuser's camera were used, and the position and course of the nerve-fibrils were determined with Zeiss's hom. imm. $\frac{1}{12}$th. For all the other figures, Hartnack, oc. 4 and obj. 7, were employed.

Fig. 1.—A vertical section of the skin of the tail of the tadpole, showing:—*a.* Corium. *b.* Basal, *c.* intermediate, and *d.* superficial cells of the epithelium. *e.* Figure of Eberth. *n.* An intracellular, *n'*, an intercellular nerve termination. *n''*. A nerve-fibril, passing through the corium and entering a figure of Eberth. Erlicki's fluid, nigrosine, saffranine.

Fig. 2.—A similar preparation, showing the epithelium and figures of Eberth highly developed.

Fig. 3.—Cells of the superficial layer of the epithelium of the tail, showing intracellular (*n*) and intercellular (*n'*) nerve terminations. Gold chloride and formic acid.

Fig. 4.—Epithelial cells of the tail, isolated after treatment with gold chloride and formic acid. *a.* A superficial cell. *b.* A cell of an intermediate layer. *n.* Intracellular nerve terminations. *e.* Figure of Eberth.

Fig. 5.—Basal cells of the epithelium, surrounded by the finely precipitated intercellular fluid. *n.* Intracellular. *n'.* Intercellular nerve endings. At *a* and *b* only the upper portions of two of the cells are seen. In the preparation from which this was drawn, the cellular protoplasm and the figures of Eberth are uncoloured and undistinguishable. Gold chloride, formic acid.

Fig. 6.—Three cells of the superficial layer of the epithelium from near the mouth, with an intra-epithelial nerve-fibril (*a*) giving off branches, one of which penetrates a figure of Eberth (*e*), two others end free between the cells; while

a fourth (b) terminates in the interior of one of the cells. Gold chloride, formic acid.

Fig. 7.—Isolated figures of Eberth, showing fine nerve-fibrils in their interior. Gold chloride, formic acid.

Fig. 8.—This represents the sub-epithelial network (a) of nerve-fibrils, as seen in and through the cells of the basal layer of the epithelium. The outlines of the cells are rendered distinct, as in Fig. 5, by the precipitation of the intercellular fluid as fine bluish granules. Three fibres (b) of the fundamental plexus, seen through the corium, give off branches terminating in beads in the epithelium, and of a considerably greater thickness than the fibrils of the sub-epithelial network. Gold chloride, formic acid.

On Green Oysters.

By

E. Ray Lankester, M.A., LL.D., F.R.S.,
Jodrell Professor of Zoology
in University College, London.

With Plate VII.

The investigation of the nature of the colouring matters which occur in different organisms, has always appeared to me one of especial importance, leading us, on the one hand, into a remarkable region of physiological phenomena, and on the other hand, helping us to trace to their true physical causes some of the most beautiful and at the same time most puzzling of organic developments. The chemical and functional characteristics of pigment-compounds are daily becoming better understood, and at the same time we are obtaining some notion of the way in which nature has here and there taken hold of the accidental non-significant property of colour in a by-product of the organism's chemical factory, and has assigned to the pigment a high position of importance as an ornament, a protective, or a lure.

In order to understand thoroughly the history of colour in the organic world we cannot afford to leave any case unexamined. The green colouring of Oysters some years ago attracted my attention because it was asserted that the colour of such Oysters was due to the taking up of copper from the seawater, brought there by the proximity of old copper mines or of copper-bottomed ships. Such a cause of green coloration in animals would have been, were it substantiated, sufficiently remarkable, both as a physiological fact and as a hitherto unrecognised mode of organic coloration, viz. by

accidental metallic impregnation. On looking further into the literature of "green Oysters," I found that though owing to certain peculiar circumstances the belief that green Oysters owe their colour to copper still survives, both popularly and among physiologists, yet it has been abundantly proved that the greening of Oysters, which is carried out as a commercial process on the coast of France, has nothing whatever to do with copper, and is directly traceable to another and perfectly definite cause, viz. the Navicula (Vibrio) ostrearia of Gaillon, on which the Oyster is made to feed.

I shall here first of all give an account of what has been ascertained with regard to the mode in which the "greening" of Oysters is produced by the agency of Navicula ostrearia. I shall then relate the curious coincidences of fact and fiction which have given support to the belief that the greening is caused by copper assimilated by the Oyster from the waters in which it lives; and, lastly, I shall describe my own observations on both the green-coloured Oysters and on the Navicula ostrearia. These observations will be found, I think, of some importance as completing the history of the mode in which the Oyster acquires its green colour, and have not only an economic or piscicultural, but also a physiological interest.

I. Discovery of the Cause of the "Greening" of Oysters.—The "green Oysters," which are known in Paris as "huîtres de Marennes," on account of the fact that they are largely brought from Marennes, on the coast of Normandy, are universally recognised as being the same species as the ordinary European Oyster (Ostrea edulis), differing only from the common Oysters in the fact that the gills and labial tentacles (and no other external part) are of a deep blue-green colour. In Plate VII, fig. 10, one of these Oysters, as seen when the right shell is removed, is represented, the drawing having been made from life in the zoological laboratory of University College, London.

In France, and some other parts of the Continent, these green-coloured Oysters have obtained a reputation for excellence, and

are accordingly in demand. The preference for green Oysters can be traced back as far as the year 1713, when it is recorded that green Oysters were served at a supper given by a certain ambassador at the Hague.

In this country green Oysters appear never to have been in fashion. They occur in some of the estuaries in Essex, but are never sent into the English market; the proprietors always export them. I have been enabled by the kindness of a gentleman connected with the Whitstable oyster trade to examine some of these English green Oysters, and I am in a position to state that they do not differ from the French "Marennes" except in being less strongly coloured than the latter.

I have not been able to find any record of green oysters differing in appearance from that figured in Plate VII. The colour is always *confined to the gills and labial tentacles*. It may be paler than that of the full-coloured specimen figured, but no *general* green coloration of an Oyster or of the Oysters from a particular bed has been recorded. This fact will be seen subsequently to have an important bearing on the question as to how the green colour is produced.

The green Oysters of Marennes do not differ in flavour from Oysters of the ordinary colour which are brought from the same locality, and there is, in the opinion of those who have made the comparison (among whom I may reckon myself), no reason, from a gustatory point of view, to prefer the one to the other.

So long ago as the year 1820 the following facts with regard to the natural history of green Oysters were made known by M. Benjamin Gaillon in the 'Journal de Physique,' tome xci, p. 222. I am indebted to an article by the late Mr. Arthur O'Shaughnessy ('Ann. and Mag. Nat. Hist.,' vol. xviii, 1866) for an account of M. Gaillon's observations, as well as for other references to the history of the subject.

Green Oysters do not occur in the sea. The green colour is acquired only in certain "parks" or reservoirs of salt water, where the Oysters are placed by the oyster merchants for the purpose of fattening and "greening." These "parks" are about 4 feet in depth and 200 feet in length by 50 feet in

breadth. From 500,000 to 600,000 oysters can be placed in each of these tanks. Tanks of this character are used by oyster merchants at Marennes, Oleron, Courseulles, Caen, Havre, Dieppe, Trèport, &c. At certain seasons of the year, particularly from April to June and again in September, the water in these reservoirs acquires a dark bluish-green tint. This is due to the growth in the tank of a particular species of Diatom—the Navicula ostrearia—which was observed with the microscope by M. Gaillon (sixty-five years ago!) and was called by him Vibrio ostrearius. M. Gaillon describes the enormous abundance of these Diatoms and their characteristic movements; he also notes their colour.

No figure of the Navicula ostrearia in its living condition, showing its beautiful combination of blue and golden colours, has, I believe, ever yet been published, and accordingly in Plate VII, figs. 1 to 9, I have given a series of coloured drawings, made from specimens which I received in the living state in London from the Director of the Botanical Laboratory of Le Croisic (Bretagne), whose kindness in supplying me with this material I desire to record. I am also indebted to the distinguished botanist, M. Bornet, for placing me in communication with this gentleman, and to my friend Dr. Vignal, of the Laboratory of General Anatomy in the College de France, Paris, for very kindly sending me on two occasions a hamperfull of "huitres de Marennes" from Paris.

M. Gaillon relates, in his memoir of 1820, that the oyster merchants carefully place the colourless Oysters dredged from various oyster-beds in the tanks where the Navicula ostrearia has multiplied to such an extent as to colour the tanks green. After a few weeks the Oysters, previously colourless, are found to have assumed a bluish-green colour in the gills and labial tentacles. If the Oyster is removed from the tanks containing the Navicula, or when the Navicula growth dies down, the Oysters gradually lose their colour, so that in the course of a month Oysters which were deeply coloured will have only the faintest trace of a green tint. This disappearance of the green colour when the Oyster is kept in

ordinary seawater I have myself observed on specimens kept in the marine aquarium of my laboratory in University College.

The opinion had been entertained by those who were not personally acquainted with the conditions of the "greening" tanks that the Oyster derived its green colour from the chlorophyll of green Algæ, upon fragments of which it was supposed to feed. M. Gaillon, however, pointed out that the green colour of these Algæ, viz. chlorophyll, was not a sufficiently permanent colouring matter to effect the coloration of the Oyster, being liable to turn yellow with age and digestion, whilst he rightly pointed to the fact that the Oyster does not feed upon coarse particles, such as these green Algæ present.

The *possibility* of the green coloration of the Oyster being due to the passage of chlorophyll unchanged from the alimentary canal of the Oyster into its blood is not apparently so remote as M. Gaillon supposed, since it results from the recent observations of Mr. Poulton, of Oxford ('Proc. Roy. Soc.,' 1885), that the green colour of the blood and integument of Lepidopterous larvæ has such an origin.

However this may be there can be no hesitation about accepting M. Gaillon's conclusion, that the *Navicula ostrearia* is the cause of the greening of the Oyster. He showed most distinctly that the *Navicula ostrearia* and the green colour of the Oyster come and go together, that where there is no *Navicula ostrearia* there is no greening, and where there is *Navicula ostrearia* the Oysters at once become green. He also showed that the Navicula has a green colour *en masse*, and that there is apparently no other green substance in the tanks on which the Oysters could feed, and so become impregnated.

For some reason which is not clear M. Gaillon's observations and inferences did not settle the question as to the cause of the greening of Oysters. There was still a belief that copper had in some way to do with the phenomenon. Possibly this is to be explained by the fact that the blue-green tint assumed by the Oyster's gills is very unlike the green colour of familiar vegetable organisms, and very closely resembles the tint of some copper salts, whilst the *Navicula ostrearia*, with its

fine blue pigment, had not been presented to the reader's eye by coloured drawings, but merely spoken of by M. Gaillon in his description of the oyster tanks.

In 1841 M. Valenciennes made an examination of the green Oysters, confining himself to the study of the green colouring matter as there seen, and ignoring the evidence brought forward by Gaillon as to the source whence the Oysters derive it.

Valenciennes drew attention to the important fact that, besides the gill lamellæ and the inner face of the labial tentacles, the liver and the intestine of the green Oyster are deeply coloured by green pigment; but the muscles, nerves, heart, reproductive organs, and blood are free from any such colouring.

Though Valenciennes did not draw the inference, it is clear that this condition points to the colouring matter being introduced into the alimentary canal, and being slowly absorbed thence and deposited in the gills and labial tentacles, the absorption taking place in such small quantity as to produce no discoloration of the blood.

It is to be noted that Gaillon had not been able to satisfy himself altogether as to the mode in which the green colour of the Navicula ostrearia became transferred to the Oyster placed in the tank with it. He discussed the possibility of the Naviculæ penetrating the gill-filaments, and rejected it; but he did not offer any proof of the swallowing of the Naviculæ by the Oyster, nor was he able to suggest how, when swallowed, the Naviculæ could impart their colouring matter to special regions only of the Oyster's body.

In a second memoir, in the 'Transactions' of the Linnean Society of Calvados, 1824, M. Gaillon suggested what appears to be the true explanation of the phenomenon, viz. that the Oyster's gill-tissue selects and deposits the colouring matter much in the same way as does the osseous tissue of pigs fed upon madder select and deposit the red colouring matter of that plant.

The observation of Valenciennes on the presence of the green colouring matter in the intestine and liver of the Oyster was therefore (though he did not know it) a confirmation of Gaillon's hypothesis.

The chief value of Valenciennes' contribution to this subject is, however, to be found in the chemical examination of the colouring matter of the green Oyster, which he carried out with the aid of Dumas, the celebrated chemist. He found that the green pigment of the Marennes Oysters was insoluble in water, in alcohol, in ether, in weak alkalies, or in weak acids; in fact it was found to be insoluble without the use of agents which destroy or fundamentally alter it, such as strong acids. He conclusively proved that the pigment did not contain copper, and M. Dumas studied it further, in order to ascertain whether it might be a compound similar to Prussian blue, and reported that it had no relation to the ferrocyanides. Accordingly Valenciennes came to the conclusion that the pigment of the green Oyster had nothing to do with metallic salts, and was due to an organic compound quite distinct from all green substances hitherto known. This, again (though its import was not recognised by Valenciennes) was a conclusion in favour of Gaillon's hypothesis, since it was thus demonstrated that chlorophyll, the pigment of the Ulvæ and common green Algæ, from which some persons supposed the Oyster to derive its green colour, had nothing to do with it.

Gaillon, however, had given no proper account of the pigment of the Navicula ostrearia, and it might well have been assumed by Valenciennes and others that the Navicula ostrearia owed its bluish-green tint to chlorophyll or to that and the water-soluble phycocyan (not properly recognised till many years later), and accordingly that this organism was excluded with all others by the peculiar characters of the pigment observed in the Oyster, from being considered as its source.

Valenciennes suggested the view that the peculiar green colouring matter which he characterised was manufactured by the Oyster itself in the intestine and liver, and was absorbed thence and deposited in the Oyster's gills.

In this condition the subject has remained[1] ever since the

[1] At Easter, 1877, I had the good fortune, in company with my friend Thiselton Dyer, to meet M. Bornet, the eminent algologist, at Le Croisic. M. Bornet subsequently sent to Mr. Dyer a dried gathering of Navicula

year 1841, with the exception of certain observations and arguments which have tended to support the erroneous theory that copper is the basis of the pigment of the Oyster. The curious history of this error I shall now relate. But I would first point out that the true history of the greening of Oysters, although brought to a certain degree of completeness by Gaillon and by Valenciennes, was still not fully worked out. It remained to show: (1st) that the Oysters do swallow the Navicula ostrearia; (2nd) that a pigment having the peculiarities determined by Valenciennes, or from which Valenciennes' oyster-pigment could be derived, actually occurs in Navicula ostrearia; (3rd) that there is some mechanism in the Oyster by which the pigment of the Naviculæ, being taken into the Oyster's alimentary canal, can be absorbed and deposited in the gills and labial tantacles, and nowhere else.

To these points my own observations have been directed, and I shall return to them immediately after giving a history of the "copper-theory."

II. The Copper-theory of Green Oysters.—It is well known to cooks and housewives that an uncleansed copper vessel is liable to impart a bright blueish-green colour to meat or vegetables which are cooked in such a vessel. The colour so imparted is very similar in tint to that of the "huitres de Marennes," and hence in the first instance has arisen the suggestion that the green Oysters have become impregnated by copper. A leg of mutton or similar material when it has acquired a green colour through the culinary misfortune above noted, exhibits a uniform distribution of the green colour. The addition of a solution of ammonia to a small fragment of the discoloured meat (even if it be only very slightly greened)

ostrearia, which, he stated, was the cause of the green coloration of the Marennes Oysters. Mr. Dyer published a note on the subject in 'Nature,' September, 1877. I have since, through M. Bornet's kind introduction, obtained the Naviculæ in a living condition. M. Bornet states that thirty-six hours is sufficient to effect the green coloration of an Oyster, previously colourless, if it be placed in a dish with a quantity of living Navicula ostrearia. He also was the first to notice (in a letter to Mr. Dyer) that the Navicula is blue and not green as Gaillon had stated.

gives the brilliant blue solution characteristic of the compound of copper and ammonia. On the other hand, the "huitres de Marennes" are not uniformly coloured green, but have only the gills and labial tentacles so coloured (see Pl. VII, fig. 10), and, moreover, the deep blue-green gill may be treated to any extent or in any way with ammonia and not a trace of blue solution can be obtained.

These facts should alone have been sufficient to cause the rejection of the popular copper-theory of green Oysters, and would no doubt long since have done so, were it not for two remarkable facts. These are:

1st. Oysters do normally contain a certain very minute quantity of copper in their blood.

2nd. Common Oysters have been stained green by fishmongers with copper-salts in order to imitate the natural green Oysters.

In reference to the first of the above statements, it is to be noted that many Mollusca and many Arthropoda have been shown by Frederiq, followed by other observers, to possess as a constituent of their blood a proteid known as hæmocyanin, into the constitution of which as much as 1 per cent. (ash) of copper enters. The detection of minute quantities of copper by Bizio (Instit. of Venice, 1845) in the tissues of Oysters may therefore be accepted. The copper so found was the copper of hæmocyanin, normally present in both green and colourless Molluscs.

The second statement as to the fraudulent staining of Oysters admits of no doubt. So far back as 1713 a case is on record, and cited by Dr. Johnston in his 'Introduction to Conchology.' A fishmonger at the Hague was ordered to supply green Oysters. Not being able to obtain any, he stained common Oysters green with copper. The persons who ate them were seized with severe colic. The fishmonger confessed his fraud.

A few years ago a similar fraud was practised at Rochefort, in France. The authors of the fraud, and those interested in maintaining the reputation of the green Oysters of Marennes, were unable to confute the evidence of the chemist, who

demonstrated in a court of law that the Oysters seized in the market of Rochefort contained copper in poisonous doses. An ingenious defence was set up. It was admitted that these Oysters were coloured green by copper, but it was asserted that they were naturally so impregnated, and that they did not come from Marennes or the coast of France at all. The French green Oysters were declared to be free from copper and harmless, but (it was stated) these poisonous coppery green Oysters had been bought in ignorance by the fishmonger from Cornish fishermen and came from Falmouth. "Now," it was argued, "it is well known that Cornwall abounds in copper, and what more natural than that a Cornish Oyster should become impregnated with that metal?" Without any evidence to prove either that there was any excess of copper in the sea-water whence these Oysters came, or that an Oyster can tolerate the presence of more copper in solution in seawater than the minute trace which is normally present, or that an Oyster or any other mollusc can take up such copper if present in sufficient quantity to colour it, the ingenious defence was admitted by the court, and the persons accused of selling poisonous Oysters were exculpated.

It would perhaps be worth while to meet the assertions of those who persist in ascribing to Oysters and Mussels a peculiar power of assimilating copper, by direct experiment. Oysters should be kept in an aquarium into the water of which a certain amount of copper-salt should be introduced, or a plate of copper inserted.

One fact which has served to strengthen the popular belief in a connection between copper and Molluscs, is the similarity of the symptoms produced by copper poisoning and by poisonous Molluscs and shellfish. Occasionally Mussels, and more rarely Oysters, and not unfrequently Lobsters and Crayfish, have produced colic, vomiting, and even death, without being green or having any history which tends to connect them with copper. The condition of the shellfish in these cases appears to be an exaggeration of a normal condition, for there are some persons upon whom Molluscs and shellfish always pro-

duce such effects. It also appears that after death a certain form of slow decomposition may occur in shellfish which develops the same poison in large quantity. It is probable that the tissues, either living or dead, develop an alkaloid in greater or less abundance which is extremely poisonous to man and to which some persons are more sensitive than others.[1] Probably the deadly-poisonous Teleostean fish which are occasionally eaten by mistake at the Cape and in Japan, owe their injurious property to the same or a similar alkaloid. When therefore we find reports of persons having been poisoned by Oysters or by Mussels which have grown upon or in the vicinity of a copper-bottomed ship (as, for instance, in the 'Edinburgh Med. and Surg. Journal,' vol. iv, p. 400), we are not justified in ascribing the colic and vomiting which such persons have suffered to the presence of copper in the Molluscs derived from the copper of the ship. It is known that Mussels and Oysters may produce these symptoms when grown apart from any special source of copper; and, on the other hand, it is NOT known that even when growing near or on copper, any Mollusc can take up into its system an abnormal amount of copper.

It is to be noted that there is no observation on record of Mussels, Oysters, Barnacles, or other marine organisms, exhibiting a green tint when removed from proximity to the copper-bottom of a ship; and, indeed, there is no evidence that any organism can live when sufficiently impregnated with copper to assume even a pale green tint.

Whilst there are so many considerations which explain the origin of the notion that copper may be responsible for the green colour of the "huitres de Marennes," although that metal has really nothing to do with it, it is extremely remark-

[1] The name "Ptomäins" has been applied to these substances which are only just beginning to be closely studied by chemists. From putrescent fish has recently been obtained a definite crystalline body, to which the name "Gadinin" has been given; and what is of extreme interest and importance is that its formation is ascribed to the action of Bacteria upon the albumens of the fish. It is probable that observers will be able to identify and isolate the particular Bacterium which produces each particular Ptomain: See Brieger "Ueber Ptomaine," Berlin, 1885.

able as a coincidence that of late years it should have been established that copper in minute quantities is a normal constituent of the blood of Molluscs.

Perhaps the strongest argument against the theory that the natural green colour of the Marennes is due to copper is (at any rate for those who do not place reliance on the results of chemical analysis) that the amount of copper sufficient to produce the deep colour seen in such an Oyster as that figured in Plate VII, fig. 10, would be so large that one dozen (not to speak of a few score) of such Oysters must infallibly cause severe symptoms of copper-poisoning in one who should swallow them. Now, though persons are sometimes afflicted with colic after eating a dozen "huitres de Marennes," the same thing has happened after eating a poached egg; and the experience of mankind is in favour of the opinion that, under normal conditions, the "huitres de Marennes" are as harmless as poached eggs.

The statements of Professor Bizio with regard to the presence of copper being connected with the green coloration of the gills of the "huitres de Marennes," deserve a little further notice. Professor Bizio has the credit of having first demonstrated (1835) by chemical analysis the presence of minute quantities of copper in the bodies of Mollusca of different genera. Ten years later it occurred to him that the celebrated French green Oysters might owe their colour to the copper which he had discovered in the ordinary brownish-grey Oysters of the Venetian lagoons. Professor Bizio never examined, and probably never saw, a true "huitre de Marennes." The whole of his essay on the subject in the 'Transactions of the Institute of Venice,' 1845, is in the highest degree imaginative. He found that the gills of a common colourless Oyster, when allowed to decompose in a glass vessel, assumed what he calls an "azure" tint. We have no measure or indication of the intensity of this azure, and we know further that Bizio, never having seen a "huitre de Marennes," was not in a position to assert, as he did assert, that this so-called "azure" colour acquired by a putrefying Venetian Oyster was the same thing as the rich blue green of

the gills of the Marennes Oyster. Bizio having noted that his putrefying Venetian Oysters turned blue, started the hypothesis that this blue colour was due to the liberation of ammonia in the tissues by decomposition of the proteids, the ammonia in its nascent condition being supposed by him to combine with the copper which he had truly and correctly determined as a normal constituent of ordinary Oysters. He (never having studied a natural green Oyster) actually proceeded further to imagine that, just as in the decomposing Oyster, a blue colour is produced by the development of ammonia and its combination with copper, so in the "huitres de Marennes," when removed from the sea and placed in tanks, a similar decomposition occurs during the life of the Oyster, and hence the gills acquire their peculiar colour. Bizio's hypothesis is entirely unjustifiable, since he did not show, in the first place, that the "azure" pigment of the putrescent Venetian Oysters was really a compound of copper. He omitted to apply the simplest tests, which might have served to establish this preliminary fact; and it is highly probable that the blue colour he noted in the course of the putrefaction of the Oysters was either an opalescence or possibly a bacterium pigment due to a micro-organism which established itself in his experimental vessels during the putrefaction.

Nevertheless we must not forget that Bizio deserves considerable credit for having discovered the presence of copper in the tissues of Mollusca at a time when the occurrence of this metal as a constituent of a living organism was a startling novelty. It was, indeed, for many years not accepted on Bizio's authority; and it is only recently that the careful study of the pigment turacin by Church from the feathers of the plantain bird, and of the blood-pigment hæmocyanin by Frederiq, Gotch and others, have definitely satisfied physiologists that copper does enter into the composition of the substances which build up animal bodies.

III. Observations on Navicula Ostrearia (Gaillon).—On two occasions I have received from the botanical laboratory

at Le Croisic, on the coast of Britanny, bottles containing a quantity of the blue-green flocculent growth caused by Navicula ostrearia. The material was in the living condition, and when a drop was examined on the field of the microscope it was found to consist chiefly of an immense abundance of a remarkable blue-coloured Navicula (the N. ostrearia), associated with a variety of other Diatomaceæ of the usual yellow-brown colour. The gatherings were obtained from tanks or "saltings" on the flat coast in the neighbourhood of Le Croisic. The Navicula ostrearia exhibited the usual to-and-fro gliding movements familiar to observers of living Diatomaceæ.

The distinctive and remarkable feature about them was the presence of bright blue pigment,[1] which appeared to be in some cases uniformly diffused through the cell-protoplasm, and in other cases to be confined to the two ends of the elongated cell-body (see figs. 1 to 9).

It is to be noted that Gaillon described these Naviculæ as uniformly impregnated with a green tint. It is hardly doubtful that this impression was due to the imperfect optical properties of Gaillon's microscope. The Naviculæ are very minute, being only the $\frac{1}{270}$th of an inch in length and the $\frac{1}{7300}$th of an inch at their greatest breadth, so that an inferior microscope might well be inadequate to enable an observer to distinguish the yellow-brown endochrome from the associated blue-coloured protoplasm, and might give a confused green appearance as the result of the combination of the two.

The yellow-brown endochrome (*c* in the figures) of Navicula ostrearia is like that of other Diatoms, and calls for no special remark. It exists generally in the form of two broad bands, which may become twisted or broken in certain conditions of nutrition and osmotic action (see figures).

The rest of the siliceous capsule is occupied by the cell-protoplasm (*d*), nucleus (*a*), and vacuoles (*g*).

[1] It will be found convenient to apply to this pigment a distinct name. I propose to call it "Marennin," in reference to the locality which has become celebrated through it.

It is important to note that the blue pigment does *not* occur as a cell-sap; does not, in fact, occupy a vacuole or vacuoles, but is diffused through the protoplasm only. In some cases it seems to impregnate uniformly the whole of the protoplasm (figs. 3 and 5), but more usually it is absent from the nucleus and the protoplasm immediately surrounding that body (fig. 1, *b*), and is confined to the protoplasm occupying the tips of the spindle-shaped organism (fig. 1, *d*).

Usually one, two, or more spherical droplets, of a more refringent nature, are to be seen scattered in the protoplasm (figs. 1, 4, 5, 6, *e*), and these appear to be more deeply impregnated with the blue pigment than is the protoplasm. At the same time it is possible that this apparent coloration of the refringent globules is an optical illusion, due to reflection of the surrounding colour.

It must be understood, in looking at the drawings (figs. 1 to 9), that each represents a particular aspect of a Navicula and a single optical plane. Thus in some the nucleus is not seen, not being in focus; in others the protoplasm is continuous, and the vacuoles are not shown owing to a superficial focussing, and so on.

All attempts to dissolve the blue colouring matter failed. Neither in bulk nor on the field of the microscope was it possible to separate the blue pigment in solution from the protoplasm of the Diatom. Weak ammonia caused the protoplasm to break up into spheres as shown in fig. 9, without parting with its blue colour. Distilled water or prolonged action of acetic acid caused a further breaking up of the blue-coloured masses into minute granules, and their total disappearance with, so far as I was able to form an opinion, the total destruction (and not the solution) of the blue colour.

The following solvents were ineffectually applied to the living Diatoms, no true solution of the blue pigment being obtained, viz. distilled water, alcohol, ether, weak alkalis, weak acids.

It is possible that a thorough attempt to obtain the blue pigment in solution from a large bulk of *dried* material, such

as could be prepared by anyone visiting one of the Normandy Oyster tanks where the Navicula ostrearia is growing in profusion between the beginning of April and the end of June, or in September, might be more successful. My observations were necessarily confined to small quantities.

IV. Comparison of the Chemical and Spectroscopic Characters of the Pigment of Navicula Ostrearia and of that of the Marennes Oyster.—The result of a comparison of the properties of the blue pigment (to be called Marennin) of the Navicula with those of the blue-green pigment in the Oyster's gills, is decidedly favorable to Gaillon's theory, though it must be admitted that the characteristics relied on are rather negative than positive.

In the first place it is important to note that Marennin is really blue and not green. When deposited in the Oyster's gill-filaments, which in common Oysters have a yellowish-brown colour, it is precisely what we should expect that the blue pigment should appear somewhat green, being in fact greener in appearance in proportion as the gill is less impregnated with the abnormal pigment, and becoming of a much bluer tint (not greener) when there is much of this pigment present.

Secondly, we note the insolubility of the pigment in both cases.

I have repeated Valenciennes' observations and can fully confirm his statements. The pigment of the green Oysters' gill cannot be dissolved by any treatment: water, alcohol, ether, glycerine, benzole, weak alkalis or acids, hot or cold, even when their action is prolonged for many hours, fail to dissolve it.[1] By strong alkali it may be destroyed, and a brown

[1] Valenciennes notes that weak acids cause the gill to pass from a green colour to blue, and that ammonia restores the green tint. This is true, but I believe is independent of any action on the special "Marennes'" pigment itself which is always blue. The change noted is due to an action on the yellowish pigment which is normally present in the Oyster's gill filaments and masks the blue pigment.

coloured solution can be obtained, but this is not a solution of the blue pigment.

Although thoroughly satisfactory experiments in bulk have not been made, there is ample ground for asserting that the blue pigment of N. ostrearia is similar to the green Oyster's pigment in resisting solvent agents.

Owing to the fact that neither pigment has been obtained in solution, there has been some difficulty in examining their absorption-spectra. That of the green Oyster's gill was examined by transmitting a powerful beam of light through a single gill lamella. No isolated absorption-bands were detected.

Similarly a mass of the Navicula ostrearia was examined by means of the micro-spectroscope and no isolated absorption-bands were noticed.

A more extended physico-chemical study of the pigment of the Navicula ostrearia is greatly to be desired; but, so far as the facts are known, they favour the supposition that the Oyster's blue-green pigment is identical with or derived from the blue pigment of the Navicula. I propose henceforward to speak of the blue pigment of Navicula ostrearia as Marennin; and I may formulate the conclusion above noted thus, viz. that Marennin derived from Navicula ostrearia taken as food is present either unchanged or slightly modified in the gills of the green Oyster, and is the cause of their colour.

V. Presence of Navicula Ostrearia in the Intestine of Green Oysters.—When Gaillon wrote, the fact that the Lamellibranchiate Molluscs feed to a very large extent upon Diatomaceæ was not so familiar, as it is to-day. Gaillon at first considered the possibility of the Navicula entering directly into the Oyster's gill filaments, and only in his second paper (Linnean Society of Calvados, 1824) came to the conclusion that the channel by which the Navicula enters the Oyster is the alimentary canal.

A very simple proof of the truth of this view which forms an

important link in the chain of reasoning by which the coloration of the Oyster's gills is connected with the blue pigment (Marennin) of Navicula ostrearia, is found in an examination of the contents of the alimentary canal of a "huitre de Marennes" when in full colour.

The examination of these contents with the microscope suffices to demonstrate that the Navicula is taken in enormous quantities by the Oyster. Not only do we remark the dark blue-green colour of the contents of the alimentary canal, but we find the siliceous shells of the Navicula ostrearia in enormous numbers.

Gaillon was at some pains to prove that Oysters do not eat floating green algæ of large size, and that in consequence the green colour of the gills of the "huitres de Marennes" was not due to the chlorophyll of such organisms.

It does not appear to have occurred to him to make a microscopic study of the contents of the Oyster's alimentary canal, which would have furnished him with a simple demonstration of the fact that the Oyster does not take in such coarse material into its alimentary canal, and does take in the Navicula ostrearia, as he inferred but did not prove by direct observation.

VI. Microscopic Appearances of the Green Oyster's Gill and Mode of Distribution of the Pigment.—Modern methods of microscopical investigation enable us to obtain a much more detailed knowledge of the Oyster's gill and of the exact position of the pigment which gives the green appearance to the "Marennes" oyster than was possible in the time of Gaillon (1820), or even of Valenciennes (1840). Valenciennes expressly states that the pigment in the Oyster's gills "n'offre rien de remarquable à l'examen microscopique." After describing the peculiar chemical properties of the pigment he arrives at the conclusion that it is "an animal matter distinct from all green organic substances hitherto studied," and further suggests that it is a peculiar modification of the bile which is assimilated, and fixes itself in the parenchyma of

the branchiæ and the labial tentacles of the Oyster "by a physiological process analogous to that which M. Flourens observed in the assimilation of madder, which gives a red coloration to the bones only of the animal fed upon it, whilst the cartilages, ligaments, and tendons remain colourless."

This reference to the observations of Flourens is not original on the part of Valenciennes, but was already made by Gaillon ('Linn. Soc. Calvados,' 1824), who, more correctly than Valenciennes, carried out completely the analogy to the case of the action of madder on bone by assigning the origin of the pigment in the case of the Oyster to a substance taken into the alimentary canal as food, viz. to the Navicula ostrearia.

We have already seen that there is abundant proof of the truth of Gaillon's view, that the pigment of the green Oyster's gill is derived from (or practically is) the pigment of the Navicula ostrearia on which it feeds, and that Valenciennes' theory as to bile is gratuitous, whilst the copper theory rests on popular fancy and the excusable mystification of Bizio, who never saw a green Oyster, but discovered the copper of hæmocyanin.

It now remains for us to examine how far the suggestion as to an analogy between the localisation of the ingested pigment in the case of the green Oyster and in the case of the madder-fed pigs of Flourens is justified.

A microscopic study of the green-coloured gills and labial tentacles of the Marennes Oyster establishes the fact that, so far from "presenting nothing remarkable" in its distribution, the green pigment is localised on the surface of these organs in certain peculiar cells of the superficial epithelium. These cells are large subspherical "secretion cells," which are placed at intervals among the smaller columnar cells which constitute the bulk of the epithelial clothing of the gills and of the labial tentacles.

The green colour is concentrated in these secretion cells, and is localised in the granules which they contain (Plate VII, fig. 14). The adoral face of the labial tentacles, when examined with a low power of the microscope, presents a dotted appear-

ance owing to the strong blue-green coloration of these cells and the colourless character of the surrounding substance.

When one of the branchial bars or filaments is isolated by teazing and examined with the microscope, it is found to present two rows on each face of these green-coloured secretion-cells, (fig. 13, *gl.*), whilst the rest of the filament is colourless.

In transverse sections of the gill (fig. 11) the curiously complicated grouping of the branchial bars is seen and the position of the secretion-cells (*gl.*). The green-coloured secretion-cells are not confined entirely in the gills to the surface of the bars, but occur also irregularly upon the internal face of the gill lamella bounding the interlamellar water spacc (*ils.*), where they are more irregularly scattered.

It is difficult to decide absolutely that there is not a minute trace of blue-green pigment diffused through the protoplasm of all the epithelial cells, but there is no doubt that the pigment is concentrated in full intensity in the secretion-cells; and I am inclined to regard the appearance of a very pale green tint diffused throughout the substance of the gill-filaments as due to optical conditions which allow the colour of those secretion-cells not in actual focus to be transferred by refraction and reflection to the surrounding colourless substance.

The secretion-cells which are thus the actual seat of the pigment in the green gills of the Marennes Oyster are now for the first time shown to play that part. They are no peculiar possession of green Oysters, but occur in exactly the same form and position, but without colour, or of a slightly brown colour, in ordinary colourless (or brownish) Oyster's gills.

The secretion-cells furnish precisely that mechanism which we should expect to find in order that the blue pigment absorbed by the blood of the Oyster from the contents of its alimentary canal, namely, from ingested Navicula ostrearia, should be deposited at a particular spot on the animal's body. These secretion-cells do not occur on other parts of the external surface of the Oyster; they are limited to the surface of the branchiæ and to the adoral surface of the labial tentacles.

Wherever they occur the green coloration occurs; where they are absent there is no green colour.

The secretion-cells are engaged in the manufacture of granules, which probably are ultimately discharged as mucin. It is a matter of some physiological interest in relation to the mechanism of the process of secretion generally to find that these cells not merely manufacture a substance like mucinogen, but actually separate from the blood a material which entered it through the walls of the alimentary canal in a condition chemically similar to that in which it is thus separated. We are not in a position to say what slight chemical modifications the blue pigment of the Navicula or "Marennin" undergoes in order that it may be rendered diffusible, and so enter the Oyster's blood. It is possible enough that it enters the blood in a condition of chemical modification which renders it colourless, and that it is only by the action of the secretion-cells that the chemical condition of the Marennin is restored in which it possesses a blue tint. Possibly the condition in which Marennin is deposited in the secretion-cells is not precisely identical chemically with that in which that body existed in the Navicula ostrearia. Possibly the Marennin retains during its passage through the Oyster's body its blue colour, but is taken up in such small quantities by the blood as to produce no visible coloration of that fluid, although its accumulation in the secretion-cells of the gills and labial tentacles renders it once more perceptible to the eye.

The fact that the Marennin is deposited in secretion-cells of the tegumentary epithelium of the Oyster, though it does not exclude a general analogy with Flourens' madder coloration of bone, yet renders it necessary to draw a marked distinction between the latter and the greening of the Oyster's gill, and to seek other analogies for the process occurring in the Oyster. For the deposition of the madder pigment is effected in a growing tissue of the skeleto-trophic group, whilst the deposit of the Marennin in the Oyster's gill is connected with the process of secretion.

It does not appear that there is any other instance on record

of a pigment introduced through the alimentary canal being eliminated by gland-cells in any part of the body in an unaltered condition, or at any rate so little altered (? milk).

The epidermic cells of the Canary separate the pigment of cayenne-pepper when the bird is fed with that substance, in such a way as to colour the feathers orange, though no other tissues are affected by the colouring matter. I have not been able to find any histological or physiological investigation of this phenomenon, which, although the mother-cells of the feather cuticle cannot be regarded as gland-cells, appears to be the nearest parallel known to the case of the green Oyster.

It is true that indigo-carmine is separated by the liver, kidneys, and other tissues when introduced into the animal body; but it is necessary to introduce the indigo-carmine directly into the blood and not through the mediation of the alimentary canal. It is also important to note that the class of secreting cells affected by indigo-carmine as well as the ready change of this body into a colourless compound, render the case presented for the study of the physiologist by the secretion-cells of the green Oyster's gill, altogether distinct and of unique interest.

Possibly the pigment Marennin might be found capable of application to the study of some of the phenomena of secretion in other animals besides the Oyster, if experimentally introduced into the alimentary canal in sufficient quantity. It would not be difficult to procure the pigment either from the Navicula (dried in large masses) or from the green Oyster.

VII. Free Amœboid Condition of the Secretion-Cells of the Oyster's Gill.—A very curious condition is commonly exhibited by the secretion-cells of the Oyster's branchial epithelium. They are to be found free on the surface of the epithelium and exhibit slow amœboid movements. At first I supposed that these liberated epithelial cells must be independent amœboid organisms, but a closer examination left no doubt that they were secretion-cells which had been detached from their position, and were leading a free wandering exist-

ence on the surface of the gill, probably on the way to disintegration accompanied by production of a mucin-like substance.

In Pl. VII, fig. 14, a number of isolated secretion-cells from the gills of the green Oyster are drawn. The upper more spherical forms were obtained by teazing; the lower figures with long pseudopodia-like processes are secretion-cells which have spontaneously assumed the free condition. The assumption of the amœboid phase by an epithelial cell is not by any means an improbable phenomenon although its occurrence in normal conditions has not, I think, been previously noted.

If it is thus possible for a constituent cell of an epiblastic epithelium to acquire amœboid characters, and to crawl over the surface of the epithelium of which it was once a constituent element, the supposition is also admissible that constituent cells of an epithelium should on acquiring amœboid characters move in the opposite direction and sink below the epithelial basement membrane, in order to enter into relation with the mesoblastic tissues. The fact observed in the Oyster's branchial epithelium suggests these possibilities, and has a value—admittedly a small one—in relation to recent suggestions as to the mechanism of the absorption of solid particles through the agency of the epithelium of the alimentary tract in higher animals.

Summary.—The new points which are brought forward in the present article bearing upon the "Green Oyster question," in addition to the general discussion of previous theories, are the following:

1. The description and illustration of "Marennin," the blue pigment of Navicula ostrearia.

2. The occurrence of Navicula ostrearia in the intestine of the green Oyster.

3. The description and illustration of the secretion-cells of the epithelium of the branchiæ and labial tentacles of the Oyster in which the Marennin absorbed in the intestine of green Oysters is deposited, and to which accordingly these parts owe their green colour.

EXPLANATION OF PLATE VII,

Illustrating Professor Ray Lankester's memoir on "Green Oysters."

Figs. 1 to 8.—Various specimens of Navicula ostrearia, Gaillon, as seen in the living condition.

Figs. 2 and 4 show that face of the specimen in which one endochrome band only is seen; the other figures give a plane at right angles to this. *a.* Nucleus. *b.* Colourless protoplasm near the nucleus (so-called "cross-band"). *c.* Endochrome band. *d.* Blue-pigmented protoplasm. *e.* Refringent spherule, apparently deeply coloured by Marennin (the blue pigment). *f.* Siliceous cell-wall. *g.* Vacuole.

Fig. 9.—A specimen of Navicula ostrearia, during the action of dilute ammonia. *h.* Broken particles of the blue-coloured cell-substance.

Fig. 10.—Specimen of a "Huitre de Marennes," of full colour. Natural size. The right shell-valve has been removed, and the right lobe of the mantle reflected so as to expose the green-coloured branchial plates and the four labial tentacles. (From a coloured sketch executed from nature by Miss A. Stone in the zoological laboratory of University College, London.)

Fig. 11.—Transverse section of a portion of a gill-plate of an oyster (green specimen), to show the arrangement of the gill-bars or gill-filaments and the position of the secretion-cells on their surface. *i l s.* Inter-lamellar water-space. *i l j.* Inter-lamellar junction. w. Aperture between neighbouring filaments, by which water passes into the inter-lamellar space. f. Gill-bars or filaments. *m f.* The "main bars" or "great filaments," interposed between projecting groups of minor filaments. *ch.* The chitinous internal skeleton of the gill-filaments. *e.* The epithelium of the inter-lamellar space. *lac.* The lacunar tissue. *gl.* The secretion cells, which are the seat of the green colour.

Fig. 12.—A transverse section of two minor gill-filaments, more highly magnified. *gl.* The green-coloured secretion-cells. *ch.* The chitinous skeleton. *b c.* Blood-corpuscles in the lacunar tissue. *n ch.* Nucleus of the chitinous skeleton. *fe.* Frontal epithelium. *l e.* Lateral epithelium. *e.* Posterior epithelium (epithelium of the inter-lamellar surface).

Fig. 13.—Portion of a single gill-bar, isolated by teazing and seen from the side, to show the rows of green-coloured secretion-cells (*gl.*). Letters as in Fig. 12.

Fig. 14.—Isolated secretion-cells from the gills and labial tentacles of a green oyster, more highly magnified. The seven upper figures as separated by teazing, the four lower figures as found naturally separated and adherent to the free surface of the gill-lamella.

The System of Branchial Sense Organs and their Associated Ganglia in Ichthyopsida. A Contribution to the Ancestral History of Vertebrates.

By

John Beard, Ph. D., B.Sc.,
Berkeley Fellow of Owens College, Victoria University, Manchester.

With Plates VIII, IX, and X.

Introduction.

Among the many weighty questions which have arisen with the rise and progress of comparative embryology, that of the origin and ancestral history of Vertebrates has occupied, and still occupies, an important place.

That the question, if capable of solution at all, would be solved by the discoveries of embryology is now, and has been for the last ten years, a general opinion among zoologists. So much for a general agreement. But as to the particular line of descent one might recall half a dozen different theories supported by different schools of workers.

The impulse to these speculations was first given by the discovery of the tadpole-like larva of Ascidians, and the opinion that Vertebrates were derived from Ascidians we owe to Kowalevski and Kupffer. This view has had its day, and is now only a reminiscence.

Another important theory, important because clothed with the authority attached to the name of Balfour, is the theory that Vertebrates arose from unsegmented worms, in which two

lateral nerve-cords were supposed to have coalesced dorsally instead of ventrally, as in Annelida.

Following this one is reminded of Hubrecht's theory, which allies Vertebrates with Nemertines, and sees the Vertebrate notochord reflected in the Nemertine proboscis sheath.

By no means least important is the celebrated Annelidan theory of the origin of Vertebrates first originated by Dohrn[1] and Semper.[2] A theory which, in spite of all attacks, still survives, and at present seems to be more probable than any other.

Finally, the alliance of Balanoglossus with Ascidians, Amphioxus, and Vertebrates, recently advocated by Bateson,[3] must be mentioned. Interesting though this is, it cannot yet be considered as sufficiently established to be accepted without reserve; but if more evidence for it be forthcoming it is a moot point whether our existing notions of the relations of Vertebrates and Annelida will not have to be modified, for we know of no existing Annelid which has relationships with Balanoglossus. And here I would point out that my own researches on the cranial nervous system and sense organs of Vertebrates, instead of supporting the alliance of Balanoglossus with Vertebrates as high as fishes, present rather a hindrance in the way of such alliance, whilst they are still more opposed to the alliance of Vertebrates with existing Annelida.

That Vertebrates have their nearest allies, except Balanoglossus, in the group of Annelida, is becoming more and more obvious from recent researches, especially from those of Dohrn; but the links of such an alliance seem to have been rather in long extinct Annelida than in any at present existing.

In the following pages an account will be given of the morphology and development of the branchial sense organs and associated ganglia in Amphibians and Fishes, chiefly in Elas-

[1] Dohrn, 'Ursprung der Wirbelthiere,' 1875.

[2] Semper, "Verwandschaftsbeziehungen der gegliederten Thiere," 'Arbeiten a. d. Zool. Institut zu Würzburg,' 1875.

[3] Bateson, W., "Development of Balanoglossus," 'Quart. Journ. Micro. Sci.,' Supplement, July, 1885.

mobranchs. The branchial sense organs are those sense organs which have usually been called organs of the lateral line, and were formerly called "segmental sense organs" by me. The name "organs of the lateral line" is bad, because it chiefly refers to those sense organs along the lateral line of the trunk, which morphologically form only a small portion of the sense organs. I have myself seen reason to reject the name segmental sense organs, because although originally they are segmental, and in later life may occur one in each segment of the trunk, still at first they are confined to one region only of the body, the gill-bearing region, and only extend into the trunk much later. Originally they are seated one above each gill cleft or over the site of each cleft, and may, therefore, be called branchial sense organs.[1]

The so-called ganglia of the posterior roots of the cranial nerves arise in connection with them, and must be regarded as originally special ganglia of these sense organs.[2]

One general conclusion may be referred to here, and that is, that at present we are acquainted with no invertebrate nervous system which is built upon the same plan as that of Vertebrates.

The matter will be discussed later on, and I only refer to it here in order that from the outset the branchial sense organs may be raised from their present position of neglect and obscurity, and may be given that important morphological (and physiological) place which their relationships to the gill clefts on the one hand, and to the ganglia of the posterior roots of cranial nerves on the other, most certainly entitle them to.

Unlike many previous observers, I have found that it is absolutely impossible to study the branchial sense organs of fishes without at the same time dealing with the posterior roots

[1] Beard, "Cranial Ganglia and Segmental Sense Organs," 'Zool. Anzeig.,' 192, 1885; also Froriep, "Ueber Anlagen von Sinnesorgane am Faciales, &c.," 'Archiv für Anat. und Physiol.,' 1885.

[2] Beard, op. cit.; Froriep, op. cit.; and Spencer, "Notes on the Early Development of Rana temporaria," 'Quart. Journ. Micro. Sci.,' Supplement, July, 1885.

of the cranial nerves, which are morphologically as well as physiologically inseparably connected with the former.

It would take up too much time and space to give here a history of all the researches on these two sets of organs, which have hitherto been usually treated apart from each other as if they had no connection.

The work has been mainly carried out on embryos of Torpedo ocellata, for which I have to thank the Zoological Station at Naples. But I have also studied Teleostei and Amphibians, and have had a few embryos of Mustelus and Pristiurus. However, in the descriptions in the following pages, unless otherwise stated, the condition of affairs in Torpedo will be understood to be under discussion.

In the first place, I think it will be of great advantage and will tend to simplify matters very much if the general schema of the development of a cranial nerve (dorsal root) of an Elasmobranch, such as Torpedo, be given.

Then those cranial nerves, which I regard as segmental, will be discussed: olfactory, nerve of ciliary segment, trigeminal, facial, auditory, glosso-pharyngeal, and vagus.

The optic nerve is left entirely out of consideration. Firstly, because I have made no investigations, and hence have no new facts about it to record; and secondly, as is well known, its whole development is different from that of the other cranial nerves; and I can only agree with those zoologists who class the optic nerve entirely apart from the other cranial nerves.

Not so, however, with the olfactory and auditory nerves and organs. Partly following Marshall, I feel bound to place these nerves in the category of cranial segmental nerves, and to class the olfactory and auditory organs[1] as specialised branchial sense organs.

Finally, after the account of the various nerves, the bearing of the facts described on the morphology and ancestral history of Vertebrates will be discussed.

[1] Beard, "On the Segmental Sense Organs, &c.," 'Zool. Anzeiger,' 161, 162, 1884; also, "On Cranial Ganglia, &c.," 'Zool. Anzeig.,' 192, 1885.

General Schema of the Development of a Dorsal Root of a Cranial Nerve.

According to the existing views of the development of a dorsal root of a cranial nerve in Elasmobranchii, based mainly on the researches of Balfour,[1] Marshall,[2] and Van Wijhe,[3] the nerve soon after its development from the neural ridge divides into two main branches, a dorsal one and a ventral one. The dorsal branch is sensory, and supplies the so-called organs of the lateral line. The ventral one is mainly motor; it soon divides again into two branches which, as Stannius[4] first showed, pass one on each side of a visceral cleft. The posterior branch is mainly concerned with the innervation of the gill muscles. According to Van Wijhe, the dorsal branch becomes intimately connected with the skin, and is there in connection with the rudiments of the so-called sense organs of the lateral line. He further holds that the sensory epithelium takes part in the formation of the nerve. In this respect the dorsal branch differs from the ventral one, which does not, according to any writer, arise either wholly or partially from the skin, but is a direct outgrowth of the neural crest (Marshall). The branch in front of the cleft is developed later than the other branches, but how is still uncertain. At any rate, both Professor Froriep and I have failed to gather from Van Wijhe, who alone has studied the development of this branch, how this branch and the Ramus pharyngeus are developed. In Amphibians Götte[5] long ago held that the so-called dorsal branches were split off from the skin.

[1] Balfour, "Elasmobranch Fishes."

[2] Marshall, "The Development of the Cranial Nerves in the Chick," 'Quart. Journ. Micr. Sci.,' 1878; Marshall, "On the Head Cavities, &c.," 'Quart. Journ. Micr. Sci.,' 1880; Marshall, "On the Segmental Value of the Cranial Nerves, &c," 'Journ. of Anat. and Physiol.,' 1882; also separate.

[3] Van Wijhe, 'Ueber die Mesodermsegmente u. die Entwickelung der Nerven des Selachierkopfes,' Amsterdam, 1882.

[4] Stannius, 'Das Peripherische Nervensystem der Fische,' 1849.

[5] A Götte, 'Entwickelungsgesch. d. Unke,' 1875.

These various branches have all received general names, some of which require alteration in view of the researches contained in this paper. The branch posterior to the cleft is called the main or posterior branch (Balfour), and post-trematic by Van Wijhe; in this paper it will be spoken of as the post-branchial nerve. The branch in front of the cleft, viz. the præ-trematic of Van Wijhe I shall call the præ-branchial nerve.

The Ramus pharyngeus of Van Wijhe will retain the same name when spoken of here. But now for the so-called dorsal branches, of all the general names this is by far the worst. It is true that the name has been employed by many distinguished zoologists, Stannius, Gegenbaur, Balfour, Marshall, and Van Wijhe, and that therefore to propose a change, except for very weighty reasons, would be a very high-handed and arbitrary proceeding. However, it must be done, and on grounds to be afterwards stated.

Though some of these various so-called dorsal nerves may come to occupy a dorsal position, still, as was first mentioned to me by Professor Dohrn, it is morphologically wrong to regard them as dorsal. Of the truth of this I have fully convinced myself, and hope soon to convince the reader also. I have, however, no means of knowing whether my reasons for rejecting the name are the same as Professor Dohrn's. These branches will be described by the general name of supra-branchial.

So much for a general view of the adult condition. A schema of the development in Elasmobranchii would be as follows. (This account is in accordance with my own researches, and contains some additions to the accounts given by my predecessors.)

The nerve grows outwards and downwards from the neural ridge towards the lateral surface of the head. In its course it lies directly under, but unconnected with, the epiblast. In the case of those nerves which are connected with gill-clefts, and are therefore typical, the nerve lies just over the cleft (fig. 50). All this is well known, and has been described by Balfour, Marshall, Van Wijhe, &c.

The subsequent events are as follows:[1]

1. When the nerve reaches the level of the notochord, or a little below that level, it fuses with the epiblast (fig. 34).

2. Part of the nerve, however, passes on to the lateral muscle-plates of the segment (figs. 34, 50).

3. At the point of fusion mentioned in 1 a local thickening of epiblast has previously taken place (fig. 14).

4. After the fusion has taken place a proliferation of some of the cells composing the thickening ensues. The proliferated cells form a mass of actively dividing elements still connected with the skin and fused with the dorsal root (fig. 16).

5. This mass of cells is the rudiment of the ganglion of the dorsal root, and externally to it is situate the rudiment of the primitive branchial sense organ of that root (figs. 12 and 13).

6. For some time cells continue to be given of from the thickened epiblast, and of those already given off many show nuclear figures (fig. 8) indicating rapid division.

7. While the ganglion is still fused with the epiblastic thickening the latter begins to grow in length and to push its way either forwards or backwards, as the case may be, between the general epiblast cells (figs. 40 and 41).

8. The general epiblast cells thus pushed away are probably lost (figs. 40 and 41, *i. e.*).

9. Concomitantly with this growth of the sensory thickening the ganglion begins to separate from the skin, and so comes to lie deeper in the mesoblast (fig. 35). As it separates there arises a nerve from the sensory thickening (figs. 11, 13, &c.). This nerve grows centrifugally from the ganglion, arising from the elements of the thickening, and being in fact split off from the latter along its whole length. It is the so-called dorsal branch, and, as previously stated, will be here called the supra-branchial branch.

10. The sensory thickening of a segment, which gives rise to

[1] Beard, "On the Cranial Ganglia and Segmental Sense Organs," 'Zool. Anzeig.,' 192, 1885; also, on some points, Spencer, "Notes on the Development of Rana temporaria," 'Quart. Journ. Micr. Sci.', Supplement, July, 1885.

the branchial sense organs of that segment, may remain very small or may increase to a very considerable length, but in any case the nerve connecting the whole length of the thickening with its ganglion is split off from the thickening, and split off simultaneously with the growth of the latter.

11. The præ-branchial nerve is also formed as the ganglion separates from the skin, and is probably in all cases also split off from the epiblast in front of each cleft.

12. Of the development of the R. pharyngeus nothing can be here recorded, but I think from the nature of the case that this nerve also probably arises from the cells on the upper wall of the cleft.

Thus, as the general result of these observations, the existing views of the development of the dorsal root of a cranial nerve will have to undergo some modification. That in Elasmobranchs the main root of the nerve is a direct outgrowth from the neural ridge, as stated by Balfour and Marshall, is certainly true. The shifting and acquisition of a secondary point of attachment described by Marshall also seem to take place. The post-branchial branch also appears to arise from the direct outgrowth from the neural ridge, but in the formation of the rest the epiblast probably plays a part. In the case of the supra-branchial branches this is certain, and it is highly probable in the case of the ganglion. That the other branches, viz. the præ-branchial and R. pharyngeus of Van Wijhe, are derived from the skin is probable, and in one case it can be proved, viz. the præ-branchial nerve of the hyoid.

Having now got a general view of the development of a typical cranial nerve, the various nerves may be considered. In the above schema we have the key to all the cranial nerves. Some, such as the ninth or glosso-pharyngeal, we shall find to fit in pretty exactly with the schema. But in others the story that ontogeny often omits or distorts ancestral history is also repeated.

Some of the branches may be absent, even in the ontogeny, while others may be abnormally developed. Others, again, may be partially fused with neighbouring nerves, as has

been abundantly demonstrated by previous writers. But whatever the adult condition of any of the dorsal roots of the cranial nerves, whatever the actual condition of olfactory nerve, nerve of the ciliary ganglion, fifth, seventh, eighth, ninth, and vagus complex, all can, by the consideration of their actual development, and of the condition of the various organs which are, or would be if present, related to them, be reduced to the general schema.

The divergences between the various nerves are, as might be suspected, naturally dependent on the presence or absence of gill-clefts in connection with the segment to which the nerve belongs.

For this reason I shall consider the nerves out of their natural order, taking those of the true gill-clefts first. Their order of treatment will thus be as follows:

Nerve.	Cleft.	Segment.[1]
Seventh.	Spiracle, and one absent.	Fourth and fifth.
Ninth.	First branchial.	Seventh.
Vagus.	Second, third, fourth, and fifth branchial.	Eighth, ninth, tenth, and eleventh.
Fifth.	Mouth.	Third.
Ciliary.	Hypophysis (?).	Second.
Olfactory.	Absent.	First.
Auditory.	Absent.	Sixth.

In the above list it will be noticed that the cleft of the fifth nerve is described as the mouth. This view, which we owe to Prof. Dohrn, seems to me to receive very considerable support from my researches. I shall refer to the matter subsequently.

For the ciliary, olfactory, and auditory nerves I have hesitated to assign clefts, because the evidence for their existence is uncertain, and the nature of the three nerves is more easily explicable if we regard the clefts as absent or metamorphosed. Here suffice it to say, that clefts have been assigned to these

[1] The numbering of the segments is in accordance with those conclusions from my researches which appear to me to be fairly certain. Probably the facial nerve is a complex of two segmental nerves, and this apart from the auditory segmental nerve. If this be the case, then there are eleven segments at least from the olfactory nerve to the fourth root of the vagus inclusive.

nerves by various zoologists, with what justification we shall see later on.

Dorsal Root of the Fourth and Fifth Segments, Seventh Nerve or Facialis.

As already described by Balfour[1] and Marshall,[2] the seventh nerve arises from the neural crest in the region of the hind brain and just in front of the auditory capsule.

These authors further agree in assigning a common root of origin for the seventh and auditory nerves. Marshall has, however, in one of his early works, drawn attention to a line of division between the ganglia of the auditory and facial nerves in the chick. Now, although the rudiments of the facial and auditory nerves lie very closely together, I consider that at first the two are really distinct. The facial grows downwards and outwards from the neural crest, and just under the epiblast. When it reaches the level of the notochord part of it fuses with the sensory thickening above the hyoid arch, and just above the future hyoid cleft. The rest passes on (fig. 20) to the lateral muscle plates of the hyoid arch. At the point of fusion with the sensory thickening the ganglion is formed. Of this, one stage is figured in fig. 20. In this condition the nerve is to be regarded as passing through an ancestral stage. Its condition is then figured in the diagram of a typical dorsal root (fig. 50), which passes from the brain to the primitive branchial sense organ and its associated ganglion above a gill-cleft, and from which ganglion a nerve passes along the posterior side of the cleft to the muscles of the gill.

In later stages the ganglion is still partly fused with the skin, but it soon separates, leaving behind it the rudiments of several branches.

These branches are the supra-branchial, the præ-branchial,

[1] Balfour, 'Comp. Embryol.,' vol. ii, p. 377.

[2] Milnes Marshall, "Head Cavities and Associated Nerves in Elasmobranchii," 'Quart. Journ. Micr. Sci.,' 1880; also, "Nervous System of Chick," 'Quart. Journ. Micr. Sci.,' 1878.

and the pharyngeal. The development of the pharyngeal branch has not yet been traced. The other branches are split off from the epiblast. The supra-branchial (figs. 21 and 22) is formed at the expense of the deeper portion of the sensory thickening, which has begun to grow forwards over the face.

Very soon this nerve divides into two branches; that is, the sensory thickening grows forwards as two divergent thickenings, and from each nerve-fibres are split off, and thus two branches are formed (fig. 51, *p. b. n.*). This development from the dichotomously dividing rudiment has been described by Van Wijhe.[1] These two branches have been described by Marshall and Spencer.[2] The upper one is the portio facialis of the oph. superficialis (Marshall), the lower one the ramus buccalis (Marshall and Spencer). The upper one Balfour, Marshall, and Spencer classed as a ramus dorsalis of the seventh. As stated by Van Wijhe,[3] they are concerned in the innervation of the supra- and infra-orbital sense organs respectively (branchial sense organs). These branchial sense organs, it is hardly necessary to state, being developed from the dichotomously dividing sensory thickening mentioned above.

The portio facialis of the ophth. superficial. (fig. 51, *p. f.*), is obviously enough, as pointed out by Marshall, Balfour, and Van Wijhe, a so-called dorsal branch; that is, what we have here called a supra-branchial. Van Wijhe has, and I fully agree with him, classed the r. buccalis (fig. 51, *r. b.*) as a "dorsal branch," and gives these reasons: (1) Its origin from the same rudiment as the former nerve; (2) its simultaneous appearance with that nerve; (3) its similar development and innervation of (branchial) sense organs. Van Wijhe, indeed, regards the two as branches of one nerve, and as therefore equivalent to one so-called dorsal branch. Dohrn[4] has advanced very weighty reasons for the

[1] Van Wijhe, op. cit., pp. 26, 27.

[2] Marshall and Spencer, "On the Cranial Nerves of Scyllium," 'Quart. Journ. of Micr. Sci.,' 1881.

[3] Op. cit., p. 27.

[4] Dohrn, "Studien zur Urgeschichte des Wirbelthier-Körpers," No. vii, 'Mittheil. a. d. Zool. Stat. zu Neapel,' vol. vi, part i.

existence of a hyomandibular segment in front of the hyoid and behind the mouth, but has not adduced the cranial nerves in support of his view. I would here venture to suggest that an additional ground for his view is to be seen in the existence of two supra-branchial nerves in the facial. It would indeed be remarkable if Van Wijhe were correct in regarding these two nerves as merely branches of one nerve, for in no other single and simple cranial nerve do we meet with more than one supra-branchial nerve. To my mind the best explanation of the presence of these two branches is that the facial is composed of the fusion of two cranial segmental nerves, and this apart from its fusion with the auditory. The reader may compare Dohrn's views on the nature of the hyomandibular with this explanation. Except for this the facial seems to be a fairly typical cranial nerve, and agrees well with the general schema. It should be noticed that the supra-branchial branches grow forwards, for this point will be referred to in discussing the vagus. Though I agree fully with Van Wijhe's[1] view that there are two segments in the hyoid arch, and this apart from the hyomandibular portion, I cannot treat the auditory nerve here. The special modifications it has undergone will be best considered after some of the other nerves have been discussed. In their earliest appearance I believe the auditory and facial nerves are not fused, and even in the later stages (figs. 21, 42), as already noticed by Marshall in the chick, the ganglia of the two nerves are partially separated, and the line of division is easily recognisable. For the later stages of the facial the reader is referred to Marshall's works and to the paper by Marshall and Spencer.

Nerve of the Seventh Segment—Glossopharyngeal.

This nerve arises from the neural ridge (Balfour) immediately behind the auditory organ. It grows down to the lateral wall of the body to just above the point of origin of the first true branchial cleft. Its fusion with the skin is represented in fig.

[1] Op. cit., pp. 9 and 28.

32, and the origin of its ganglion from the skin and in connection with the branchial sense organ of this segment in fig. 42. The main portion of the nerve grows downwards behind the cleft, and proceeds to the lateral muscle plates of the first branchial arch.

Later, as the ganglion separates from the skin, the supra-branchial nerve is developed. Like other supra-branchial nerves, it splits off from the skin in connection with a sensory thickening which gives rise to the supra-temporal sense organs.

Marshall described the course but not the development of this branch in the embryo.

The direction of growth of this nerve is somewhat different from that of the corresponding branches of the seventh. It grows dorsally and forwards (fig. 51, *s. t. g.*).

In late stages præ-branchial and pharyngeal nerves are developed, but I have no observations as to their mode of origin to record.

It is obvious that the glossopharyngeal agrees exactly with the general schema. The sole peculiarity to be noticed is the direction of growth of its supra-branchial branch. As in the cases of other nerves, the shifting and secondary attachment described by Marshall probably occur; I have, however, not studied them.

Nerves of the Eighth, Ninth, Tenth, and Eleventh Segments—Vagus complex.[1]

The actual development of this complex has been fairly accurately described by Van Wijhe. However, as in the cases of other nerves, he omitted to record some steps in the process of development, and referred the actual connection of the complex with the skin to a later stage to that in which it first arises.

He further, though describing the connection of the supra-branchial branches with the skin, and though figuring the

[1] For the vagus the condition in Torpedo is taken, in which there are at least four nerves concerned; in Hexanchus the vagus has five elements, in Heptanchus six (Gegenbaur).

actual fusion of the vagus ganglia with the sensory thickening, does not ascribe to the skin any part in the formation of the ganglia.

Like Van Wijhe, I cannot find in the vagus outgrowth itself any real segmentation in its earliest stages. The first outgrowth from the neural crest (fig. 33) is a broad uninterrupted band stretching from just behind the glossopharyngeal, which it almost joins, to a considerable distance backwards.

Like other posterior roots, this outgrowth grows outwards and downwards towards the portion of epiblast just above the second, third, fourth, and fifth branchial clefts, which are now just forming (fig. 33). Here the epiblast forms a longish sensory thickening, with which the vagus fuses.

Portions of the vagus pass on (fig. 34) behind the rudiments of each of the above-mentioned clefts, and form, as in other cases, the post-branchial nerves.

At the point of fusion with the skin, cells are proliferated from the epiblast to form the ganglia.

Soon, as pointed out by Van Wijhe, we get the ganglion of the first vagus cleft separated from the rest of the mass and fused with an isolated thickening above the second true branchial cleft.

For the rest of the vagus there is usually only one ganglionic mass, which, however, ventrally, and by its post-branchial branches, shows a division into three portions. This mass lies over the last three clefts, and is to be regarded as made up of the fused ganglia of the three branchial sense organs of these clefts, with the addition, however, of rudiments of nerve elements of a certain number of clefts, which have disappeared; and even in the ontogeny hardly present traces of their former existence. In Torpedo, however, as first noticed by Wyman,[1] there is a rudiment of one cleft which never breaks through to the surface, and hence which is never functional.[2] The rudi-

[1] Wyman, "Observations on the Development of Raja batis," 'Mem. Amer. Acad. of Arts and Sciences,' vol. ix, 1864.

[2] This paper of Wyman's was not accessible, and the statement in the text is given from Balfour's 'Embryology,' vol. ii.

ment of this cleft is very obvious in horizontal longitudinal sections of certain stages, and is represented in fig. 47. Here there is a considerable hypoblastic depression (*cl.* VI) of the pharynx just behind the last or fifth branchial cleft.

Corresponding to it is a shallower but still marked epiblastic involution. Along the posterior side of this hypoblastic depression the intestinal branch of the vagus runs. Gegenbaur has regarded this branch of the vagus as containing rudiments of post-branchial branches of aborted clefts; and I think that in the relationship of this intestinal branch in Torpedo to rudiments of a sixth cleft we have a new support for his view.

The ramus intestinalis is, as Van Wijhe states, mainly made up of the post-branchial branch of the last true visceral arch; but, as just stated, it must also contain portions of the post-branchial branches of one or more aborted clefts. Certainly this is the case in Torpedo.

In the question of the homology of this nerve I can only agree with Van Wijhe in rejecting Balfour's view that the ramus intestinalis is a commissure.

The statement just made concerning aborted clefts is also in accordance with Van Bemmelen's researches on the thymus. His discovery of thymus elements behind the vagus is mentioned by Dohrn[1] in his last great work, as supporting his view that Vertebrates formerly possessed many more gill-clefts than they do at present. The question will be returned to later on.

It is thus seen that in Torpedo at any rate the vagus contains the elements of at least four segmental nerves and the rudimentary portion of a fifth.

The first one of the lot is, shortly after its first development, slightly separated from the fused mass which contains the sense organs and ganglionic portions of the rest.

Hence vagus 1 can be treated alone. As mentioned before, its post-branchial branch passes along the posterior wall of the second branchial cleft to the musculature of the cleft. The skin

[1] Dohrn, "Studien zur Urgeschichte, &c.," No. vii, 'Mittheil. a. d. Zool. Stat. zu Neapel,' Bd. vi, Heft 1.

takes no part in its formation. Above the cleft the main nerve fuses with the skin, and there as in other cases ganglion and primitive branchial sense organ are formed. In this case too —and fig. 34 shows it fairly well—the sensory thickening must be considered as taking part in the formation of the ganglion.

Later, the ganglion separates from the skin, and, along with this separation, the sensory thickening grows forwards and takes also a dorsal direction, a supra-branchial nerve splits off, and the sense organs formed are part of the supra-temporal branchial sense organs (fig. 51, *st. v.*). Here as in the glosso-pharyngeal, the supra-branchial branch has a dorso-anterior direction.

Vagus 1 also fits into the schema very well. It is formed just in the way described in the schema, has the same relation to a cleft, develops a primitive branchial sense organ and associated ganglion, &c. In fact, its development might have been taken in giving the schema.

For the rest of the vagus there is only one ganglionic mass, and one long, broadish thickening with which the ganglionic mass is associated.

When the common nerve rudiment grows from the neural ridge and fuses with the epiblast, at the point of fusion the ganglionic mass is proliferated, probably entirely from the skin. From the ganglionic mass branches are sent off along the posterior sides of each of the three last clefts to the musculature of the clefts. They are the post-branchial branches, and are not developed from the skin. The last of the three is the so-called intestinal branch of the vagus. Along with the separation of the ganglion from the skin, the sensory thickening begins to grow backwards along the lateral surface of the trunk (fig. 39). This thickening is the rudiment of the so-called lateral line. The description of its development to be given here is in the main identical with that given by Van Wijhe.[1] It agrees with Götte's[2] and Semper's[3] researches

[1] Op. cit., pp. 34, 35.

[2] Goette, 'Entwickelungsgesch. d. Unke.,' p. 672.

[3] Op. cit., p. 256.

in so far as it describes the origin from the skin of the so-called lateral nerve, and in this point it differs from Balfour's account.[1] It is, as Semper stated, very easy in Elasmobranchs, though by no means so in Teleostei, to follow the whole development of the lateral line and nerve.

In horizontal longitudinal sections the whole process is obvious enough, and I can fully endorse Van Wijhe in the opinion that Balfour would have had no doubt about the matter had he studied the point with horizontal sections instead of with transverse ones. The question of the direction of sections is here a vital one. In (fig. 39, *vg. gl.*) the compound vagus ganglion is represented as fused with the skin, and the lateral line, *l. l.*, has commenced to grow backwards.

It is an interesting, and by no means an unimportant point, that the lateral line increases in length not by the actual conversion of the epiblast cells behind the growing point of the line into sensory cells similar to those already present in the line, but that there is an actual growth backwards of the lateral line itself (figs. 40 and 41). That is, the sensory cells which compose the rudiments of the "line," and which anteriorly give rise to the compound vagus ganglion (*vg.* 2, 3, and 4), repeatedly and rapidly divide, and in such a manner that the "line" is increased in length and pushes its way between the indifferent epiblastic cells behind it (fig. 40). These indifferent epiblastic cells (figs. 40 and 41, *i. e.*) are actually thrust aside and probably lost along the whole course of the "lateral line" and concomitantly with its growth.

Part of the epiblast which is cast off is figured in figs. 40, 41, *i. e.* It is possibly this temporary epiblast seen in transverse section which led to Balfour's view of a special origin of the canals of the sense organs in the trunk of Elasmobranchs.

As in other cases the nerve of the sense organs, the so-called lateral nerve, is formed from the deeper portion of the sensory thickening. This mode of origin of the lateral nerve was first

[1] Balfour, 'Elasmobranch Fishes,' p. 141.

described by Semper, and afterwards more fully by Van Wijhe in Elasmobranchs.

The point is far easier to determine here than in the cases of other supra-branchial nerves, indeed, it attracts the eye with startling distinctness in horizontal longitudinal sections of embryos of the proper age. The nerve is formed as the sensory thickening grows backwards along the body. It is well shown in figs. 40 and 41, *l. n.*, and can be traced from the vagus ganglion (*vg. gl.*) backwards along the thickening, gradually becoming thinner and less differentiated until finally it ceases in the cells of the sensory thickening.

That here there is no actual growth backwards of the nerve is obvious enough, for when the development has taken place for some length, then near the ganglion the nerve is fibrillar and has few nuclei, these latter increasing as the nerve proceeds backwards, and the fibres becoming pari passu, fewer, and ending gradually in the protoplasm of the sensory thickening.

Where the compound vagus ganglion (*vg. gl.* 2, 3, 4) separates from the skin (fig. 36) it is easily seen that above each of the three branchial clefts, viz. the third, fourth, and fifth branchial clefts, fibres are given off from the separating ganglion to the sensory thickening. In fact, each of the elementary nerves making up the vagus compound, viz. *vg.* 2 and 3, and the intestinal branch, *vg.* 4 and 5, takes part in the formation of the so-called "lateral line." In other words, the lateral line is made up of supra-branchial nerves of at least four segmental nerves, probably of more than four, viz. vagus 2, 3, 4, and 5. The fifth root is the rudiment of the nerve of the rudimentary cleft mentioned before.

We have seen that the facial, which is probably a compound nerve, has a large forked supra-branchial branch, and we shall find that the fifth and ciliary also, as already well known, have each a very long supra-branchial nerve, extending over the snout (fig. 51, *op. s.* and *oph. pro.*), and hence we need not be much surprised that a supra-branchial nerve, which is made up of the elements of at least four supra-branchial

branches, should grow right away to the tail, and supply a very long series of branchial sense organs.

In a former note[1] I put forward certain hypotheses concerning the posterior roots of spinal nerves to account for the apparently abnormal innervation by the vagus, that is, by a cranial nerve complex, of a region extending right to the tail. These hypotheses I now see reason to reject, and after a study of the actual facts of development in Elasmobranchs, as now recorded, I can only conclude that the so-called lateral line only differs in length and direction of growth from the other branchial sense organs. Its length is sufficiently accounted for by its containing the elements of at least four supra-branchial nerves, and its direction offers in itself nothing really remarkable, for the direction of growth of the other supra-branchial branches is not always the same. Those of the fifth, seventh, and ciliary grow forwards; those of the glosso-pharyngeal and vagus I grow dorso-anteriorly, and that of the rest of the vagus grows backwards (figs. 46 and 51).

In fact, the direction of growth of sense organs and nerves would seem to be determined by the usefulness or need of having branchial sense organs in regions of the body other than the region just above the gill-clefts where they primitively occur.

Judging by the great variations one meets with in the arrangement of these branchial sense organs in Ichthyopsida it would seem as though different families of fishes and Amphibians had independently solved the matter for themselves. The great morphological point to be noticed, and I shall lay great stress on it later, is that at first there is the rudiment of one branchial sense organ with its associated ganglion over each gill-cleft or over the site of a potential gill-cleft.

With reference to the hypotheses about spinal nerves mentioned above, I may here state that I see no reason now for assuming that true spinal nerves were ever connected with

[1] Beard, "On Segmental Sense Organs, &c.," 'Zool. Anz.,' 161, 162, 1884.

branchial sense organs. So far as my researches go there is a wide difference both in morphology and development between the cranial and spinal nerves.

The mode of development of the lateral nerve here described is, as previously mentioned, in the main the same as that ascribed to it by Van Wijhe. The only author who has assigned to it a different origin in Elasmobranchs is Balfour, who was inclined to the view that the nerve really grows backwards from the vagus ganglion.

My own researches on Teleostei[1] led me to accept Balfour's view, but since I have had the opportunity of investigating the matter in Elasmobranchii I conclude that my interpretation of the matter in Teleostei was erroneous.

No doubt the account given by Hoffmann[2] of the development in Teleostei is correct. It accords well with the facts as recorded for Elasmobranchs here and by Van Wijhe.

But none the less, it may not be superfluous to point out that the existing accounts of the development of what I have called supra-branchial nerves in Teleostei, Elasmobranchii, and Amphibians—that is, the accounts given by Semper, Götte, Hoffmann, and Van Wijhe, contain in them one element of uncertainty. That is, as to how the nerve thus developed from the skin acquires its connection with the appropriate ganglion.

Most of the accounts are quite silent on this point; Götte, it is true, recognised the importance of the matter, and stated that the nerve in any particular case separates from the skin along part of its length and grows to its ganglion. This view, however, is not in accordance with the facts, and I have reason to believe that Prof. Götte has now himself ceased to hold it.

The apparent absence of connection between the nervous structures of the brain and the branchial sense organs of the head was to Balfour a great objection to Götte's and Semper's view. He said, and to a certain extent he was right, that at

[1] Op. cit.

[2] Hoffmann, "Zur Ontogenie der Knochenfische," 'Archiv für Micros. Anat.,' Bd. xxiii, p. 45.

first there is no nerve in connection with the developing sensory thickening.

This is right so far as its growing point is concerned, for there the nerve has not developed.

But, as Van Wijhe has pointed out, it is not really the case so far as relates to entire absence of nerve in connection with the sensory thickening, and, further, the connection between sense thickening and nerve is best made out in early stages, and is afterwards not so easy to trace.

Van Wijhe himself, though he has given a true, accurate, but somewhat incomplete account of the development of these supra-branchial branches to the sense organs, cannot be said to have solved the difficulty under discussion. He has rather ignored it, and though possessing the material for its solution has not mentioned the matter. It is very curious that, although he has figured the fusion of various ganglia with the skin, he has apparently not noticed that the supra-branchial branches grow in the various cases out of the various ganglia so fused, and therefore are in connection with their appropriate ganglia from the first.

In fact the whole rationale of the formation of supra-branchial nerves is to be seen in the deploying of the branchial sense organs, and in the connection of these organs with the ganglionic centre by longer or shorter conducting fibres—the supra-branchial nerves. Originally the sense organs were restricted to one over each gill-cleft with an associated ganglion.[1] This increased, and gave rise to two by division, and so on. This is the more certain when we remember that even in late stages, according to Malbranc,[2] the sense organs of Amphibia increase by division. I have myself noticed and recorded this mode of increase in embryonic Teleostei.[3]

[1] Beard, "Segmental Sense Organs and Associated Ganglia," 'Zool. Anz.,' 192, 1885; also Froriep, "Ueber Anlagen von Sinnesorgane am Facialis, &c.," 'Archiv für Anat. und Physiol.,' 1885.

[2] Malbranc, "Von der Seitenlinie u. ihren Sinnesorganen bei Amphibien," 'Zeit. f. wiss. Zool.' vol. xxvi, 1876.

[3] Beard, "Segmental Sense Organs of Lateral Line," 'Zool. Anzeiger,' Nos. 161, 162, 1884.

It is hardly necessary to repeat that Gegenbaur's view of the composition of the vagus out of a number of typical posterior roots is quite true. We have seen that it really contains rudiments of at least five such elements in Torpedo.

It follows from this that the vagus agrees with the schema given in the preceding pages. It is equivalent to, and shows the development of, at least four such schematic nerves. True there is only one supra-branchial branch,[1] the lateral nerve, for all the elements of the vagus except the first. But this is probably secondary, and due to the fusion of the posterior elements of the vagus, and, as stated before, *vg.* 2, 3, and 4, all give fibres to the lateral line.

It is worth mentioning here, because these researches confirm one of Balfour's views, that the "lateral line" was originally, as he believed, restricted to the anterior part of the body. The whole development of all these branchial sense organs shows the truth of this. But it is, at the same time, a very curious fact that these sense organs along the trunk of Teleostei are segmental (fig. 44, *br. o.*). This is well known, and is figured in the above figure, which is part of a horizontal section of a salmon hatched about six weeks.

At one time I believed with Eisig and others that great morphological importance could be attached to this fact, but I feel now compelled to adopt Balfour's view, and in discussing the morphology of these sense organs I shall strongly urge that in face of the facts of development here recorded, the morphological connection between these branchial sense organs of Vertebrates and the "Seitenorgane" of Capitellidæ, first suggested by Eisig,[2] becomes of a very doubtful nature. And here again I may be permitted to remind the reader that Balfour[3] long ago rejected the existence of any homology between these two sets of organs.

[1] In Torpedo and many other forms. In other cases the "lateral line" is more complicated; especially is this the case in Amphibia, vide Malbranc, op. cit.

[2] Eisig, "Die Seitenorgane der Capitelliden," 'Mittheil. a. d. Zool. Stat. zu Neapel,' vol. i. 142.

[3] Balfour, 'Comp. Embryol.,' vol. ii, p. 142.

Vagus in Amphibia.

Mr. Spencer has recorded in this Journal[1] certain observations on the nerves of Amphibians. He has found that not merely the ganglia of the dorsal roots of cranial nerves of Amphibians, but that the whole of the nerves themselves are split off from the skin. I have figured the origin of the vagus nerve and ganglion in the frog in fig. 27. I have investigated the facts in Amphibians, and can fully confirm Mr. Spencer in most points. The development as seen in Amphibians is interesting, as in some respects showing a very primitive condition of the nervous system, viz. a nerve sheath or part of one; in other respects it is impossible in them to get as good a view of the primitive nerve composition of the head as in Elasmobranchs.

In Amphibians a considerable amount of fusion of once separate nerves has taken place, not only behind the auditory organ, but also in front of it. As an instance, it may be mentioned that the ciliary ganglion, which in Elasmobranchs, and even in birds, is quite distinct in its development, is in the Amphibians fused with the Gasserian, and the two arise together as one fused mass.

Vagus 1, 2, 3, and 4 are also all fused into one mass in Amphibia; the figure (27) is a transverse section through this mass. In it the nerve has not separated from the skin, and the ganglionic portion is readily recognisable as a mass of yolk-filled cells on the level of the lateral line. Later, both ganglion and nerve leave the skin as in Elasmobranchs.

Nerve of the Third Segment—Trigeminal less Ophthal. Profund.

The fifth nerve is well suited for studying the development of the ganglion of a dorsal root.

It is well known, from Balfour's and Marshall's researches (*opera cit.*), that it arises from the third of the brain vesicles.

[1] 'Quart. Journ. Micr. Sci.,' Supplement, July, 1885.

In fact, from their researches and those of Van Wijhe, the development of the fifth is fairly well known with the exception of three stages. These are, the fusion with the skin, the formation of the Gasserian ganglion, and the mode of development of the supra-branchial nerve (portio minor of the ophthal. superficialis, Schwalbe).

To explain these stages it will be necessary to repeat some facts which are already known.

The outgrowth from the neural ridge, which forms the rudiment of the fifth, is broad and extends backwards almost to the region of the seventh. Anteriorly it stretches forwards almost to the region of the ciliary to be hereafter mentioned.

But the region between the two ganglia is well defined in the earliest stages by the indifferent epithelium between them, and by the position of the second head cavity which lies between them (fig. 11, *h. c.* 2).

The nerve rudiment grows down to the level of the notochord (fig. 14), and fuses with an epiblastic thickening, just as the other nerves do. Here cells can be seen leaving the thickening to form the ganglion (fig. 15).

In this case and in that of the ciliary there can be little doubt as to the actual mode of formation of the ganglion. The thickening which gives rise to the ganglion is situated just dorsad of the mouth, and in fact has just the position of a branchial sense organ.

The ganglion is figured in fig. 17, still connected with the skin, and possessing then what we may regard as its primitive branchial sense organ.

Later, the sensory thickening grows in an anterior direction, and as it does so the ganglion separates from the skin, leaving behind it, as in other cases, a nerve, which is split off from the sensory thickening, and which is the supra-branchial branch of the fifth (fig. 51, *op. s.*). Its course, &c., have been described by Marshall and Spencer, and it is usually called the portio minor of the ophthal. superfic. It was first classed as the r. dorsalis of the fifth by Balfour, and Marshall and Spencer afterwards expressed their agreement with this view. Where the

main nerve fuses with the skin its course is continued along the mandibular arch by a number of cells of the nerve. These form the post-branchial branch, and innervate the musculature of the mandibular arch. Later, a præ-branchial nerve is developed (Van Wijhe and others), which hooks over the angle of the mouth in the way that other præ-branchial branches hook over gill-clefts.

Another apparent branch of the fifth is the nerve which Marshall has called a communicating nerve between the ciliary and Gasserian ganglia (fig. 51, *c. b.*). Its true nature has been worked out by Van Wijhe, who has shown that it really belongs to the ciliary ganglion. As I accept this statement I shall describe the nerve, as Van Wijhe has done, as part of the nerve of the second segment.

The ophthalmicus profundus (fig. 51, *oph. pro.*) is also a part of the nerve of the second segment; this has been recognised by Marshall and Spencer, and also by Van Wijhe.

The later fusions which occur between the fifth and seventh and the fifth and ciliary are in the early stages absent. In fact in its development the fifth has the typical characters of the posterior root of a gill-bearing segment. It fulfils in every way, as Marshall found, the requirements of a segmental nerve as laid down by him, and it accords with our schema. It possesses a primitive branchial sense organ and an associated ganglion just above a cleft, the mouth. It has the homologues of post-branchial and præ-branchial branches, and it develops a supra-branchial nerve in connection with the branchial sense organs over the snout (fig. 51, *op. s.*).

The new additional light thrown on the nature of the mouth will be referred to in discussing the general morphological considerations arising out of these researches. Suffice it here to say that the facts given above seem to me to confirm Dohrn's[1] conclusion that the mouth arose from a pair of coalesced gill-clefts.

[1] Dohrn, "Studien, &c.," No. 1, 'Mittheil. a. d. Zool. Station zu Neapel,' Bd. iii, p. 252.

Second Segmental Nerve—Ophthalmicus Profundus, Ciliary Ganglion, and Radix Longa.

A good deal of confusion exists as to the actual nerve components of this segment.

Marshall[1] regards the motoroculi as the main stem of the ciliary ganglion, and attributes to it the character of an anterior and posterior root. In Marshall and Spencer's[2] paper the ophthalmicus profundus is also classed as part of this segment. Schwalbe[3] had previously shown that the ciliary ganglion was really the ganglion of the posterior root of this segment, a demonstration which Marshall confirmed embryologically. Following on and extending these discoveries Van Wijhe recognised the most important component of this segment in the ophthalmicus profundus, which he classed as the posterior root of the segment. While accepting to a certain extent Van Wijhe's view, the writer feels bound to admit that from Van Wijhe's researches alone, the matter does not stand in a very clear light.

Here, as in other cases, Van Wijhe's preconceived notions as to the correspondence of the roots of cranial nerves to those of spinal nerves, interfered with the proper interpretation. Marshall[4] first gave an account of the development of the ciliary ganglion; this account Van Wijhe added to, but it is still by no means complete. And although the development of no cranial ganglion is easier to follow and no fusion with the epiblast more obvious than the development and fusion of the ciliary ganglion, this fusion has never before been figured, and Van Wijhe's earliest stage figured (fig. 31, *gl. c*, op. cit.) is a stage at which the ganglion is in great part separated from the skin, and in which the ophthalmicus profundus which runs from the

[1] Marshall, "Segmental Value of Cranial Nerves," 'Journ. of Anat. and Physiol.,' 1882.

[2] Op. cit., p. 29.

[3] Schwalbe, 'Das Ganglion Oculomotori.'

[4] Marshall, "Head Cavities and Associated Nerves, &c.," 'Quart. Journ. Micr. Sci.,' 1880.

ganglion along the snout and forms the supra-branchial branch, has just begun to develope.

A glance at the diagrams (figs. 45 and 46) of the cranial nerves, according to the writer's views, will simplify matters and pave the way for the account shortly to be given.

Taking the ninth nerve, or glossopharyngeal, as a type of a cranial nerve to a true gill-cleft, we see that there is a main stem (*p. r.*), a ganglion with associated sense organ, and then three other branches. These are—a post-branchial (*p. n.*), a præ-branchial (*p. b. n.*), and a supra-branchial (*s. b. n.*). As their names imply, the post-branchial and præ-branchial run behind and in front of the cleft respectively. The supra-branchial nerve is the nerve connected with the later developed additional branchial sense organs.

Now we may turn to the nerve of the second segment. The first thing noticeable is that the cleft is absent,[1] or at any rate the gill muscles are not present even in the ontogeny.

As a natural corollary to the absence or metamorphosis of the cleft, and absence of its muscles, the post-branchial and præ-branchial nerves are also aborted.

In the diagram this abortion is represented by dotted lines (fig. 46). Hence all that we can expect to find of the posterior root of this segment is a supra-branchial branch to the branchial sense organs, the ganglion of the branchial sense organs, and the main stem connecting the ganglion with the brain. The ganglion is the ciliary, the main stem is the radix longa, connecting the ciliary and Gasserian ganglia, and the supra-branchial branch is the ophthalmicus profundus.

This identification is very similar to that given by Van Wijhe, but the matter is approached from an entirely different point of view.

[1] Or metamorphosed. Dohrn has recognised what he believes to be a cleft behind the nose and in front of the mouth in the hypophysis. He does not say that it is the cleft of the ciliary ganglion, but this would seem to follow if Dohrn's view were accepted. As at present, though possible, no relationship of this supposed cleft to the ciliary ganglion has yet been demonstrated, Dohrn's view must be accepted with reserve.

The actual development is as follows: From the neural crest of the midbrain, just before the closure of the neural folds, cells grow outwards and downwards to a thickened patch of epiblast just above and behind the eye (fig. 7).

This outgrowth has been seen and described by Marshall and Van Wijhe. But Marshall recognised in it the first rudiment of the motoroculi, and Van Wijhe that of the ophthalmicus profundus. Neither observer saw the skin fusion or the development of the ganglion. When the outgrowth reaches the thickened patch of epiblast it fuses with it (fig. 6). Cells are then proliferated off from the skin to form the ganglion, and the outer portion of the thickening begins to form the primitive branchial sense organ (figs. 8 and 9). From the thickening cells are given off for some time until a large ganglionic mass is formed, which still for some time remains fused with the skin.

In fact, in the case of the ciliary ganglion the mode of development is well marked and very easy to study. The sensory thickening soon begins to grow forwards over the snout, and as it does so the ganglion begins to leave the skin. As this takes place a nerve is developed from the thickening, and connects the ganglion with its branchial sense organs.

From its course, relations, &c., this nerve is seen to be the ophthalmicus profundus.[1] It is morphologically the supra-branchial nerve of the second segment.

The distance between the ciliary and Gasserian ganglia, even in early stages, is very short. The outgrowth from the neural ridge which forms the main stem of the ciliary ganglion is practically continuous with the outgrowth which forms the main stem of the fifth. Van Wijhe has also drawn attention to this.

Hence it can hardly be wondered at that the connection of the two ganglia with the brain soon becomes a common one, which distally divides into two portions, one of which is continued on to the Gasserian ganglion, while the other goes

[1] Apparently also Van Wijhe's indentification, but not very obvious from his description.

somewhat obliquely to the ciliary, and forms its so-called radix longa (fig. 51, *c. b.*).

Although I have no observations to record as to the development of the third or motor-oculi nerve, still Marshall's opinions on the nature of the nerve must be discussed, and as his views are inconsistent with the other facts as recorded in this paper, I shall state what seem to be urgent reasons for modifying them.

Marshall has advanced the suggestion that the third and fourth nerves together make up a segmental nerve. He says,[1] "There is very strong reason for thinking that, in the chick at any rate, the third nerve develops, like the hinder cranial nerves and the posterior roots of spinal nerves, as an outgrowth from the neural crest on the top of the midbrain." Since the third nerve later on arises from the base of the midbrain, "very near the mid-ventral line," he infers that the nerve must shift downwards, and to an extent unequalled by any other nerve.

Now, leaving aside the fact that the shifting in the case of the third nerve, if it does take place, occurs, by Marshall's admission, to a greater extent than in the case of the other cranial nerves, a point which is surely of some importance, there are other objections which cannot, I think, be ignored. Marshall's views have also been contested by Van Wijhe, for whose reasons the reader is referred to the oft-quoted work on the nerves of the Elasmobranchii.

In any discussion as to the nature of the third nerve the morphology of the head cavities is bound to have an important place. The second or mandibular head cavity undoubtedly gives rise to the superior oblique muscle (fig. 12, $h.\ c._2$). On this point I can fully confirm Van Wijhe.

This fact alone ought to dispose of the fourth nerve, which Marshall considers as part of the nerve of the second segment, that is, as part of the third nerve. The mandibular head cavity arises from the mesoblast plate of the mandibular

[1] Marshall, "Segmental Value of Cranial Nerves," 'Journ. of Anat. and Physiol.,' p. 35, 1882.

arch, according to Balfour, Marshall, and Van Wijhe. It gives rise to the superior oblique muscle, therefore the nerve of this muscle, the fourth nerve, must also belong to the mandibular segment, as Van Wijhe insists.

Further, if the first head cavity is morphologically of the same nature as the second and third head cavities, then the third nerve, which innervates the muscles derived from the first head cavity, is, a priori, of the same nature as the fourth and sixth nerves.

Marshall himself regards the sixth nreve as a ventral root of the seventh nerve,[1] and says, "Concerning the actual value of the sixth nerve, I see no reason to alter the opinion I previously expressed, that the sixth nerve may be regarded as having the same relation to the seventh that the anterior root of a spinal nerve has to its posterior root."

We have also seen reason to believe that the fourth is a ventral root of the trigeminal nerve. And from all these facts we might fairly regard the third as also a ventral root.

But further, the dorsal root of no other cranial nerve, if we except the third, innervates the structures arising out of a head cavity. The dorsal roots, so far as they are motor, only innervate those structures derived from the lateral muscle plates (Van Wijhe).

According to Van Wijhe, the third nerve develops after the ciliary ganglion, and hence could not be its dorsal root. The third, at any rate, is an exceedingly fine nerve, and is much thinner than the ophthalmicus profundus; hence, if the third nerve be the dorsal root of the second segment, then the proximal stem of the nerve is thinner than one of its distal branches. Hence there seems to be no avoiding the conclusion, in which I agree with Krause and Van Wijhe, that the third is not the dorsal root of the ciliary ganglion, but is the ventral root of the second segment.

Returning to the general schema of the development of the dorsal root of a cranial nerve, it is found that, so far as its development goes, the nerve of the second segment agrees with

[1] 'Segmental Value, &c.,' pp. 42—44.

the schema. In this instance allowance has to be made for the absence of a gill-cleft, and, more especially, of a gill musculature. In this the absence even in the ontogeny of post-branchial and præ-branchial branches is accounted for. Otherwise the development is normal. There is a main stem with primitive branchial sense organ and an associated ganglion, the ciliary. There are no other branches except the later developing supra-branchial nerve (ophth. profund.). This nerve, as elsewhere, is developed in connection with the extension forwards of the branchial sense organs (fig. 51 *oph. pro.*). The reduction which has probably taken place in the nerve of the second segment prepares the way for the recognition and interpretation of the still greater specialisation which the two remaining cranial segmental nerves have undergone. It affords a better insight into the true nature of the olfactory and auditory nerves.

First Segmental Nerve—Olfactory Nerve.

The olfactory nerve has usually been classed with the auditory and optic nerves apart from the true segmental cranial nerves.[1] Dohrn, in his essay on "Die Ursprung der Wirbelthiere," first suggested that the nose was a gill-cleft, and Marshall[2] very strongly advocated this view as the result of his researches on the chick and in Elasmobranchii. He insisted, and as I believe with justice, on the segmental nature of the olfactory nerve. His reasons for this view were based on the actual development of the olfactory nerve; and he states—and so far as my researches go they only confirm his statement—that "the olfactory nerve develops in precisely the same way as the cranial (segmental) nerves;" they arise at first from the upper part of the forebrain and gradually shift downwards, acquiring by so doing a secondary connection with the cerebral hemispheres, of which they are at first completely independent; and

[1] Huxley, 'Anat. of Vertebrates,' p. 71; Gegenbaur, 'Elements of Comp. Anat.,' English trans., p. 515; Götte, 'Entwickelungsgesch. d. Unke, &c.'

[2] Marshall, A. M., "The Development of the Cranial Nerves in the Chick," 'Quart. Journ. Micr. Sci.,' 1878, p. 23; and also, "Morphology of the Vertebrate Olfactory Organ," 'Quart. Journ. Micr. Sci.,' 1879.

finally, the olfactory lobe or vesicle, so far from being the earliest part to be developed, is actually the last, no vestige of it appearing in the chick until the seventh day of incubation, in the salmon till long after hatching, or in the dogfish until stage O of Balfour's nomenclature."[1]

For the rest it is hardly necessary to repeat here the evidence advanced by Marshall of the segmental nature of the olfactory nerve, though in the writer's opinion not quite conclusive, it is of value so far as it goes, and it will be summarised later on after additional evidence has been adduced in favour of the segmental nature of the olfactory nerve.

But Marshall recognises in the olfactory organ the rudiment of a gill-cleft, and, as I am led to a somewhat different view, it may be of advantage to give a summary of Marshall's reasons for this opinion.

For the detailed account the reader is referred to the paper on "The Morphology of the Vertebrate Olfactory Organ." The following abstract is taken from Wiedersheim's 'Lehrbuch der Vergleichenden Anatomie,' p. 375. The epitome there given is so concise and clear that I do not feel it necessary to offer any excuse for reproducing it here.

Starting from the fact that the olfactory nerve agrees in its development with the other cranial nerves, that is, that it represents a spinal-like nerve which springs from the neural ridge, Marshall regards the olfactory groove as a primitive gill-cleft, which in exactly an analogous position to that in which the true gill-clefts are supplied by branches of the glosso-pharyngeal and vagus, has an anterior (upper) and a posterior (lower) branch of the olfactory nerve, these branches being respectively in front of and behind the supposed olfactory cleft. The Schneiderian folds of the nasal mucous membrane are comparable to the gill-filaments of fishes. As a consequence of the above view a communication between the nasal and oral cavities must once have existed in all Vertebrates, including fishes. Leaving aside the fact that such a condition is still present in Myxinoids, traces of it are to be seen in the naso-

[1] Marshall, 'Segmental Value of Cranial Nerves,' p. 13.

oral groove of Selachians, and also in the development of other fishes. Thus Marshall found in salmon embryos obvious diverticula of the oral mucous membrane, which stretched towards the nasal groove, but which later in the development disappeared. Smelling, argued Marshall, is only a modified breathing, and thus no violent physiological change is necessary to convert a gill into a smelling organ.

Wiedersheim[1] himself formerly supported Marshall's view, and pointed out that in Epicrium, and probably in other Gymnophiona as well, there are on either side two olfactory nerves, one dorsal and one ventral, the roots of the two being perfectly independent, and some little distance apart. He considered these roots to be homologous with the dorsal and ventral roots of a spinal nerve, and that by their discovery the segmental rank of the olfactory nerve was established. But, as Prof. Wiedersheim has kindly informed me by letter, he has, since the appearance of Blaue's paper ("Ueber Bau der Nasenschleimhaut bei Fischen und Amphibien," 'Archiv für Anat.,' 1884), seen reason to change his views on this subject.

The contents of this really important paper will be referred to shortly, and here I need only express my conviction that the results of Blaue's work taken in conjunction with the light which I hope to throw on the development of the nose and its relationship to the other branchial sense organs, settle in a very definite and satisfactory manner the true homology of the nose.

What has now to be demonstrated is that the nose is really a branchial sense organ, that is, the sense organ of a non-existent gill-cleft, and not a gill-cleft itself.

It ought here to be mentioned that Hoffmann has already expressed a very similar view of the nature of the nose.[2] That is, he compares its whole development to that of the ear and

[1] Wiedersheim, 'Anatomie der Gymnophionen,' 1879, pp. 59, 60.

[2] Hoffmann, "Zur Ontogenie der Knochenfische," 'Archiv f. Micros. Anat.,' Bd. xxiii, p. 88.

of the so-called organs of the lateral line, and rejects Marshall's view entirely.

Although I have very little that is new to add concerning the development of the olfactory nerve, still the novel way in which its development will be regarded is not without importance.

It was seen in discussing the nerve of the second segment—the root of the ciliary ganglion—that the whole nature of the nerve of this segment was obvious enough when it was noticed that the musculature of the lateral plates, that is, the gill musculature, was absent, even in the ontogeny.

As a consequence post-branchial and præ-branchial nerves were absent, and the whole segmental nerve was reduced to a ganglion and a supra-branchial sensory nerve. This nerve, as its name implies, being connected with the innervation of the still existing branchial sense organs. Of course the main stem of the nerve connecting ganglion and brain was also present.

A very similar condition of things exists in the nose. The early development has its exact parallel in the development of the nerve of the second segment. The sole difference is that the sense organs of the nose have not, as in the case of those of the second segment, undergone further development in a linear direction (fig. 46) but have confined that development to a somewhat circular area. That is, they have developed in many directions, but to a limited extent in each. A change of function has also probably occurred. In higher forms, this, of course, is certain.

A glance at the diagram (fig. 46) will illustrate the meaning of the above remarks. The supra-branchial nerve of the second segment (*s. b. n.*) is represented by a line. In the nose (*olf. o.*) a supra-branchial nerve can hardly be said to be present. The sense organs have developed within an enclosed figure.

For the rest, the development of the nerve of the first seg-

ment is practically that of a typical segmental nerve in which post and præ-branchial branches are aborted.

The nerve grows down from the brain to a thickening of epiblast, it fuses with this thickening (fig. 1), and a ganglion is formed at the point of fusion (figs. 2, 3 and 4). Even with the limited amount of material at the writer's disposal, it can fairly well be shown that the ganglion is formed from the skin.

When the nerve first fuses with the skin, just as in other cases, no ganglion is present (fig. 1).

The ganglion first develops after the fusion, and from the inspection of figs. 2, 3 and 4, which are camera drawings of actual sections, it will be plain that there are strong reasons for believing that, as in other cases, the ganglion is proliferated from the sensory thickening. At any rate, in a later stage, which has also been figured by Dr. Marshall (fig. 5), it is seen that the state of affairs here exactly resembles that in the ciliary ganglion and thickening (fig. 8), Gasserian ganglion and thickening (fig. 17), &c. The only difference between the olfactory ganglion and thickening and the complete segmental nerve, ganglion, and thickening of a gill-bearing segment, is the absence in the olfactory segment of any præ- or post-branchial nerves.

Fig. 2 shows us a ganglion fused with an epiblastic sensory thickening and connected with the brain by a short nerve stalk. In fact it is the picture of a branchial sense organ and its associated ganglion.

The facts of development here given, which accord so marvellously with the development of the other cranial segmental nerves, certainly render necessary a modification of Marshall's view as to the nature of the olfactory organ, and in fact a modification in the sense of the above passage, in which the nose is regarded not as a gill-cleft, but as the sense organ of a gill-cleft.

Marshall based his views firstly on the correspondence in anatomical and histological structure between the nose and other gill-clefts, secondly, on the frequent occurrence of two branches of the olfactory nerve, one on each side of the sup-

posed cleft; and he further compared the Schneiderian folds of the nasal mucous membrane, as Stannius[1] had previously done, to the folds of a gill.

The facts of development, as stated by Marshall, have been here admitted, but at the same time slightly extended, and in such a wise that the development of the olfactory nerve and organ were shown to agree very closely with the nerve, ganglion, and branchial sense organs of any other cranial segmental nerve.

But now as to the relationships of the branches of the olfactory nerve to the supposed cleft, and as to the nature of the branches themselves.

In its earliest development the olfactory nerve shows nothing that can really be homologised with the post-branchial branch of a cranial nerve. Such a resemblance, when present at all, is only existent in much later stages.

But the post-branchial branch of a cranial nerve, whenever developed, is *par excellence*, concerned with the innervation of the gill musculature, and if it contains sensory fibres its main portion is motor. There is nothing like a gill musculature, even in early stages, connected with the olfactory organ.

No one has yet described an arterial arch, gill cartilage, or musculature, in connection with the supposed nasal visceral arch. The Schneiderian folds have indeed, in Elasmobranchii and other forms, a certain resemblance to gill folds, but this alone would not be sufficient to homologise the two structures, and the folding could be more easily explained as brought about by the mere physiological need of increased surface. But surely it is a great change from a respiratory structure and function to a sensory structure and function. A change which, in spite of the basis of truth in Dohrn's law of change of function, has not, so far as the writer is aware, been shown to have occurred in any other case. True, Dohrn[2] has recognised a

[1] Stannius, 'Lehrbuch der Vergleichenden Anatomie,' ii Theil.

[2] Dohrn, "Studien, &c.," No. 2, 'Mittheil. a. d. Zool. Stat. zu Neapel,' Bd. iii.

gill-cleft in the hypophysis, but he has declined to ascribe a sensory function to that structure.

Froriep[1] also, in discussing the writer's views as to the nature of the Vertebrate auditory organ, has suggested that the ear is really a modified gill-cleft. But, as I shall presently show, this suggestion cannot be accepted, or even be held with any amount of reserve, for it is based on erroneous ideas of the primitive nature of the dorsal roots of cranial nerves.

If the writer's discoveries stood alone, he would conceive it as highly probable, if not certain, that the nose is really a branchial sense organ. But this view of its nature is confirmed in a most striking manner, and rendered as certain as anything can possibly be by the researches of Blaue.[2]

These researches have been carried out on a considerable series of fish and Amphibians, and have led to the conclusion that in the lowest form of adult nose met with, viz. the nose of some fishes and Amphibians, Belone, the herring, and Proteus, the structure of the nasal membrane is essentially made up of a series of "smell buds" (Riechknospen) and between these an indifferent stratified epithelium. These smell buds are identical in structure with the so-called taste buds of the papilla foliata of the tongue, say of a rabbit, and are also identical with the structures in the skin of fishes, which are here called branchial sense organs, and which are usually known as sense organs of the lateral line.

In the common Triton those structures described by Blaue are readily found in transverse sections passing through the nasal cavities. One such section is figured in outline in fig. 48, and a part of the section, showing two sense bulbs of the nose or smell buds, is figured under high magnifying power in fig. 49.

In Triton I have fully convinced myself by actual investigation that Blaue's results are true and accurate. And I have also somewhat examined the state of things in a few fishes. There

[1] Froriep, "Ueber Anlagen von Sinnesorgane am Facialis," 'Archiv für Anat. und Physiol.,' 1885.

[2] Blaue, "Ueber Bau der Nasenschleimhaut bei Fischen und Amphibien." 'Archiv für Anat. und Physiol.,' 1884.

can really be no doubt as to the accuracy of Blaue's results; and here it only remains to give a very short resumé of the paper, referring the reader who desires further detail to the original, which is illustrated by a number of very beautiful drawings.

In many Amphibians and fishes the nasal membrane has the structure mentioned above, but in others the indifferent epithelium becomes reduced, so that the bulbs come to lie nearer together. This reduction of the indifferent epithelium begins around the bases of the buds. The basal epithelium is pushed away, and in such a fashion that basally the bulbs are in contact, but are separated by indifferent epithelium distally (Exocoetus).

In Trigla typical smell buds are found along with such as have increased in width and pushed the indifferent epithelium away.

In Cottus the smell buds are almost completely fused together, but there is still a little indifferent epithelium, and a few buds still remain isolated.

Lastly, in Fierasfer and others the indifferent epithelium has disappeared entirely from the folds of the nasal membrane, and a continuous sensory epithelium is present.

Thus Blaue has furnished very valuable evidence, from which in conjunction with our knowledge of the development in Elasmobranchii the nature of the nose can be decided with greater probability than hitherto.

In Elasmobranchii separate bulbs are not present even in the embryo. The indifferent epithelium has disappeared even in the ontogeny, but from Blaue's researches on the structure of the nasal membrane in adult fishes generally, and from the mode of development of the nose, its ganglion and nerve, there can really be no hesitation about classing the nose with the branchial sense organs, and hence we are justified in calling it the modified sense organ of a gill-cleft.[1] F. E. Schultze[2] had

[1] Beard, "Cranial Ganglia and Segmental Sense Organs," 'Zool. Anz.,' 192, 1885.

[2] F. E. Schultze, "Ueber die beckerförmigen Organe der Fische," 'Zeit. f. wiss. Zool.,' Bd. xii, 1863.

previously stated his conviction that the "Geschmackorgane" or taste buds were the last remains of the skin sense bulbs of fishes, and Blaue now homologises the smell buds and the sense bulbs of the skin of fishes.

But though he is convinced of this homology, he nowhere hints that the nose is to be regarded as a specialised portion of the so-called organs of the lateral line, and in fact accepts and supports Marshall's gill theory of the nature of the nose, and derives his smell buds from skin sense bulbs which, originally present on the nasal visceral arch as in other cases, have wandered into the nasal cleft.

Now, although sense bulbs are present on and along the visceral arches of many fishes, they are not primitively there, their primitive position being above the cleft, not along it. Their presence along the arch is a later development. This fact and the facts of development as given before are entirely opposed to Blaue's supposition.

It is a curious commentary on the influence of the same set of facts on the views of different zoologists that while Blaue, as the result of his researches, advocates the gill nature of the nose, Prof. Wiedersheim, as he has kindly informed the writer by letter, since reading Blaue's paper, considers it necessary, as most morphologists would, to give up entirely the notion that the nose is a gill-cleft.

My own opinion does not rest on the researches of Blaue alone. Apart from those discoveries, I should believe myself justified in holding, as against the views of Prof. Dohrn and of my own teacher, Prof. Marshall, that the nose is the modified sense organ of a gill-cleft rather than a gill-cleft itself.

But though maintaining that Blaue's results are not necessary to support this view, yet, blending together those results and the facts recorded in this paper as to the development, &c., of the supra-branchial sense organs and of the nose itself, I believe that my view of the nature of the nose has so solid a foundation in facts that even the most sceptical zoologist can have little hesitation in accepting it.

Shortly stated, the olfactory organ is a branchial sense

organ, and the olfactory nerve is a segmental nerve, the post-branchial and præ-branchial branches of which, in consequence of the absence of a nasal cleft, are not developed. In fact, the olfactory nerve is the sensory remnant of the most anterior segmental nerve.

Development of the Nose in Amphibia and Teleostei.

Hoffmann has described the development in Salmo, but has not ascribed an epiblastic origin to the nerve; this, however, is the case in both Teleostei and Amphibians. In Amphibia Götte held that the olfactory nerve was developed in mesoblast. In fig. 4 the developing olfactory nerve and organ of a Teleostean Rhodeus amarus is figured, and in fig. 3 a similar stage in Rana temporaria. In both cases there is an epiblastic thickening, with which is united the rudiment of a ganglion, and there is also the rudiment of a nerve, the future olfactory nerve (*olf. n.*), just splitting off from the skin. The development here is precisely similar to the development of the fifth nerve in the frog as described by Spencer, or to that of the vagus in the same animal as described in the preceding pages.

It is hardly necessary to say that these facts confirm what has been said of the nature of the nose in Elasmobranchii.

Nerve of the Sixth Segment—Auditory Nerve.

In a former paper[1] I suggested the homology of the auditory organ with the so-called organs of the lateral line or branchial sense organs. Subsequent investigation has only confirmed this suggestion.

Gegenbaur originally ranked the auditory nerve as a dorsal branch of the seventh. On embryological grounds Marshall and Balfour had also been led to the conclusion that the auditory nerve was not in itself entitled to segmental rank, but was in its development only a dorsal sensory branch of the

[1] Beard, "On the Segmental Sense Organs, &c.," 'Zool. Anzeig.,' Nos. 161, 162, 1884.

seventh. Marshall, indeed, held that there was not room for another segmental nerve between the seventh and ninth.

Recent researches have led different zoologists to the opinion that the hyoid arch is composed of two originally distinct arches.

Van Wijhe considers that the obliterated cleft was behind the facial nerve, while Dohrn holds that it was in front of the hyoid cleft. The possibility that both are right appears to me not unlikely. Dohrn sees remains of a former cleft in the hyo-mandibular and in the thyroid body. The only evidence afforded by the nerves in support of this appears to me the existence of two supra-branchial nerves for the seventh. Alone it is not convincing evidence, but taken in connection with Dohrn's facts[1] it is, I think, of importance.

That a cleft formerly existed behind the hyoid cleft and in front of the first branchial is not admitted by Dohrn, and he has declined to attach any weight to the reasons which Van Wijhe urged for this opinion, which was based on the presence of two head cavities in the hyoid arch. Van Wijhe does not appear to have attached much importance to the evidence offered by the nerves, for he did not regard the auditory nerve as in itself of segmental value, and he never suggested the homology of the auditory organ with the branchial sense organs.

Development of the Auditory Nerve.

In Elasmobranchii the facts of development for this segment are exactly comparable to those described for the olfactory segment. The arrangement is here the same. There is no gill-cleft, and of course, as a consequence of the absence of that, we cannot expect to find a post-branchial nerve.

[1] Dohrn even goes further, and postulates a separate spiracular visceral arch just behind the mandibular arch. Thus, according to Dohrn, there are four arches included between the fifth nerve and seventh nerve, viz. mandibular, spiracular, hyomandibular, and hyoid. So far as my researches extend, I have found nothing in the nerves that would suggest a spiracular arch. However, bearing in mind what has taken place in the case of the vagus, I should hesitate to cast even a doubt on the truth of his view.

The following line of argument may, as in the case of the olfactory, be used for the auditory segment. The sense organs and ganglion connected with the ciliary segment are without doubt homologous with the sense organs and ganglion of a cleft-bearing segment such as the glossopharyngeal. The ciliary has no præ- or post-branchial nerves because no gill-musculature or cleft. The auditory segment has no præ- or post-branchial branch just as the ciliary, but its sense organs, ganglion, and nerve are just exactly like, and have the same structure as the sense organ, ganglion, and nerve of the ciliary segment. Therefore the auditory nerve, organ, and ganglion are homologous with the nerve, sense organ, and ganglion of the ciliary segment, and therefore are also the homologies of the nerve, sense organ, and ganglion of the glossopharyngeal segment. But the sense organ and ganglion of the latter are a branchial sense organ and its ganglion, therefore the auditory organ is also a branchial sense organ, and the auditory nerve the remnant of a segmental nerve.

Immediately behind and somewhat overlapping the sensory thickening which gives rise to the facial branchial sense organ is a long and broad auditory thickening (fig. 23). Behind the outgrowth of the neural crest which forms the facial nerve there is at a certain stage a small short outgrowth, this is the rudiment of the auditory nerve (fig. 23). It soon reaches the auditory thickening, fuses with it (figs. 24 and 25), and the ganglion begins to be formed at the point of fusion, and probably from the thickening itself as a proliferation just as in other cases.

Before the auditory involution has proceeded very far there is a considerable ganglion formed, and fused with the auditory thickening (fig. 29). At this stage the whole nerve, sense organ, and ganglion correspond exactly with the nerve, sense organ, and ganglion of the ciliary segment (fig. 8).

Soon the involution is carried to such an extent that the auditory organ forms a sac, but it still opens on to the surface, and in Elasmobranchs remains so throughout life. Even after the formation of the sac cells continue to be given off from the thickening to form the ganglion (fig. 31). The later formed

semicircular canals, &c., are obviously secondary complications, which have as their motive the extension and perfection of the sensory surface, and which resemble somewhat the formation of a supra-branchial nerve and its sense organs.

The resemblance in structure between the sensory cells of the ear and those of the branchial sense organs is obvious enough, and need not be dilated upon here.

In Amphibia (Rana temporaria) the auditory organ, nerve, &c., are formed just like the sense organ, nerve, &c., of the trigeminus of the same animal. The nerve is split off from the epiblast, the auditory thickening is developed from the deeper layer of the epiblast opposite the notochord, and, as in the stage figured (fig. 28), there is no auditory ganglion, it is fair to assume that it is formed just as in other cranial posterior nerves in Amphibia in connection with the auditory thickening.

In Elasmobranchii, &c., the auditory ganglion and nerve become so fused with the facial that the nerve has usually been described as a branch of the facial. We have seen that it developes separately from the facial, and even when partially fused (fig. 21), the line dividing the two nerves is readily seen (also Marshall).

General Considerations.

Morphology of the branchial sense organs.—It is pretty clear from the facts recorded in the preceding pages that the so-called organs of the lateral line have some physiological relationship with the gill-clefts. They arise at the same time as the latter, are originally seated one over each gill-cleft, and have each a ganglion of a dorsal root of a cranial nerve arising with and attached to them. From the ganglion nerve-fibres pass to the gill musculature, on the one hand, and to the brain on the other. In fact, these sense organs may very well be regarded as special sense organs of the gill-clefts or as branchial sense organs. This conclusion Prof. Froriep and I have independently arrived at.

From the above and from the facts of development recorded

here, it also follows that the ganglia of the posterior roots are primitively ganglia of these branchial sense organs. Originally connected directly with its branchial sense organ, the ganglion of a posterior root has now left its primitive position and has come to lie in the mesoblast, being only connected with its sense organ by nerve-fibres. In this conclusion as to the nature of the ganglion I am again independently in agreement with Froriep and Spencer.

In describing the schematic development of a dorsal root I have I think sufficiently emphasized its true nature. Primitively a dorsal root of a cranial nerve is the nerve of a gill-cleft, and is apparently only connected with the innervation of its cleft. It sends fibres from the brain to the sense organ and ganglion above the cleft, thence other fibres pass to the musculature and walls of the cleft (fig. 50).

It is not without importance to notice that any division of the dorsal root of a cranial nerve into so-called dorsal and ventral branches is primitively absent (fig. 50). Such divisions only occur in the later development in consequence of the separation of the ganglion from the skin, and of the formation of a greater number of branchial sense organs. Of course the ventral branch is there from the start, but in itself it is mainly motor and gives rise to no ganglion, and probably never has sense organs in connection with it. It certainly is not directly concerned in the innervation of a primitive branchial sense organ. Through a misunderstanding of this point Professor Froriep has been led into rather serious errors as to the nature of the dorsal roots. He concluded from Van Wijhe's researches, and I must admit, not without reason, for the matter is there very vaguely stated, that the branchial sense organ and ganglion could occur on the ventral branch of a cranial nerve as well as on a dorsal. This conclusion led him to the opinion that the auditory nerve is a ventral branch. The blame of the matter lies very much at the door of Van Wijhe, for he described a cranial nerve (dorsal root) as typically possessing two branches, a dorsal and a ventral one, both of which could possess a ganglion. Now, we have seen in

the development that the so-called dorsal branch (supra-branchial nerve) forms late in the development, and arises solely by the necessity of extension and increase of the branchial sense organs, with which it is solely concerned, the ventral branch as such is probably solely concerned with the innervation of the gill-clefts.

A few words may be devoted to the researches of Bodenstein[1] and Solger,[2] which have led to the conclusion that in the sense organs of the lateral line in Teleostei nerve strands connecting the various sense organs together are present. From the account of the development given here such a connection might be expected to occur, for I have shown that the "lateral line" has arisen solely by the extension and multiplication of the primitive branchial sense organs of the vagus; they are, as we have seen, connected in development, being formed from one continuous sensory rudiment, and as they form one physiological whole we could expect a connection in the adult. Although I have not attempted here to give an account of the development of the "lateral line" in Teleostei, I may perhaps be allowed a few words on it as it seems to confirm the researches under discussion.

In this case in the growth backwards of the sensory rudiment there are found thicker portions, which are segmental, and thinner portions connecting them. The nerve is split off along the whole length, just as in Elasmobranchs. The thicker portions give rise to the sense organs, the thinner portions only to nerve structures, and probably to those connecting strands described by Boderstein and Solger.

Remains of Branchial Sense Organs in Higher Vertebrates.

Prof. Froriep's paper, leaving aside the small error just mentioned, is a very interesting and very important addition

[1] Bodenstein, E., "Der Seitencanal von Cottus Gobio," 'Zeit. f. wiss. Zool.,' Bd. xxxvii, Heft 1.

[2] Solger, "Ueber die Seitenorganen Ketten der Fische," 'Zool. Anzeig.,' 1882, No. 127, p. 660.

to our knowledge of the ancestry of Mammalia. It is mainly concerned with the description of rudiments of these branchial sense organs of the facial, glossopharyngeal, and vagus in Mammalia, viz. cow and sheep embryos. These rudiments are only found in certain stages and disappear later. When they still exist the corresponding ganglia of these cranial nerves, viz. the ganglia of facial, glossopharyngeal, and vagus, are fused with the skin, indeed, the conditions seem to be much the same as in Elasmobranchii. That the ganglia are wholly or partly derived from the skin in Mammalia Prof. Froriep hesitates to decide. It is somewhat remarkable that Prof. Froriep should have failed to find rudiments of such sense organs in connection with the Gasserian and ciliary ganglia, and I cannot help expressing a firm conviction that such rudiments exist at some stage or other in Mammalian development. This conviction rests on a twofold basis, an *a priori* one that in Elasmobranchii the sense organs of the ciliary and Gasserian ganglia are very well developed, and secondly, on the discovery, of which I hope soon to give a full account, that *such rudiments occur, and are very obvious in embryo chicks.* They are in the chick especially obvious in the cases of the ciliary and trigeminal segments, but they also occur in the segments of the facial, glossopharyngeal, and vagus.

Of course here, as in Mammalia, they disappear after the fish stage has been passed through, but when they attain the maximum of their development one could almost fancy in studying them that it was an Elasmobranch embryo which was under examination. The state of affairs in both cases being so alike that one can only marvel that these rudiments have hitherto escaped notice in the chick. So much for the present.

The Nose and Ear as Branchial Sense Organs.

In the preceding pages abundant evidence has, I think, been adduced to show that the nose and ear are specialised branchial sense organs. Whether they ever had gill-clefts in connection with them is a point which, from the evidence at present at

our disposal, we cannot decide, and can only suspect that such was once the case from the relationship of the other branchial sense organs to gill-clefts, and from the known facts that certainly Vertebrates once possessed more clefts than at present. At any rate, at present the thymus or thyroid of the nose and ear, or their equivalents, have still to be found.

The only zoologists who have suggested a different view of their nature are Froriep and Blaue, who have suggested that the ear is a gill-cleft. Apart from the evidence given in the preceding pages, which is inconsistent with this view, one may reasonably ask that the supporters of such a view shall give us more evidence than that afforded by an epiblastic depression that an organ is a gill-cleft.

In this matter the nose and ear stand on equal terms, and until we have a few more of the structures which compose a gill-cleft and visceral arch, such as arterial arch, cartilage, &c., assigned to them, we can reasonably regard the matter with a certain amout of reserve.

It is interesting to notice that if my views be correct the nose and ear are the only remains of the branchial sense organs[1] in the adults of higher Vertebrates. They have survived with a possible change of function, while the other branchial sense organs have disappeared except in the first stages of the embryo, and are then only transitory structures.

The Morphology of the Suprabranchial Nerves.

This point has, I think, been sufficiently demonstrated in the general part of this work. The supra-branchial nerves are merely concerned in extensions of the branchial sense organs to a distance from the ganglia. They are erroneously called dorsal, for this condition when acquired is purely secondary.

Any commissural nature of some of these branches, as suggested by Marshall and Spencer, is out of question. None of

[1] Professor F. E. Schultze notwithstanding, the possibility that the taste buds of the tongue of higher Vertebrates are also to be referred to those sense organs must be borne in mind. Their innervation by the glossopharyngeal is, in this connection, very suggestive.

them are remains of the neural ridge. Still less can I accept Spencer's recent suggestion,[1] that "the two curious branches which unite respectively the fifth and seventh and fifth and third cranial nerves" . . . "may be regarded as persistent parts of the lateral nerve which united the ganglia of the sense organs along the lateral line in the head, and which, separating from the skin, have come in the course of development to occupy a much deeper position, together with the ganglia, with which they preserve their primitive connection."

These "curious branches" are portions of fused suprabranchial nerves, as a glance at the diagrams (figs. 46 and 51) will show.

The Relations of the Head and Trunk in Vertebrates.

Many attempts have been made to homologise the components of the segments of the head and trunk, and naturally such attempts have extended to the nerves. The spinal nerves, it is hardly necessary to say, present an anterior and posterior root, and the posterior one is ganglionated. Such a state of affairs has been sought for also in the head, but in face of the facts previously recorded it is at least doubtful, even if the evidence of cranial anterior and posterior roots be granted, whether these can be homologised with those of the spinal nerves. The posterior roots of cranial and spinal nerves develop differently, for the spinal have no connection with the skin in early stages; that is, the ganglion is never fused with the skin, and their roots are never connected with gill-clefts or with special sense organs.

One of the most striking results of these researches is the great distinction of the body of Vertebrates into a gill-bearing region, and a non-gill-bearing region; and at present, with the sharply-defined differences which obtain in the development of the organs of these two regions, attempts to homologise organs in the two different regions would seem to meet with indif-

[1] Spencer, 'Notes on the Early Development of Rana temporaria,' p. 12.

ferent success. That Balfour was right in regarding the cranial nerves as more primitive than the spinal is probable enough, but at the same time it is very questionable whether the spinal nerves ever had the same primitive characters as the cranial.

Dohrn's idea that the anus arose from a pair of coalesced gill-clefts may be rejected without more ado, for there seems to be no evidence for it. Not so, however, his mode of regarding the mouth as a pair of coalesced gill-clefts, that is probably true. In dealing with the relations of head and trunk the vexed question of anterior roots of cranial nerves crops up, and with it the nature of the head cavities. I have no observations to record on the so-called anterior roots of cranial nerves except on the hypoglossus, which has certainly nothing to do with the cranial nerves, as Dohrn has pointed out. Van Wijhe regarded the hypoglossus as made up in Elasmobranchs of three anterior roots of the vagus. In this point my researches agree with those of Dohrn and Froriep. The hypoglossus has nothing to do with the vagus.

Froriep's[1] account of the development of the former in Mammalia seems to hold good also for Elasmobranchs. As in Mammalia the hypoglossus of Elasmobranchs is derived from the anterior roots of the first three spinal nerves. The posterior roots are developed in the embryo, but afterwards abort. I have not figured them, because the spinal nerves really lay beyond the scope of this work.

As to the head cavities themselves, their persistence in the anterior part of the head may, as other observers have stated, be due to their functional connection with the eyes, that they once occurred in all the segments of the head is probable enough, though with what organs they were originally connected is not so plain. Possibly from their muscular nature, and the apparent absence of sensory elements, even in development, in their nerves, they may have been the muscles of neural parapodia. That they had nothing to do with the gill-clefts themselves is pretty certain.

[1] Op. cit., pp. 5 and 48.

Nature of the Mouth.

A few words may be here said on the bearing of these researches on the nature of the mouth.

Dohrn[1] first suggested that the mouth was primitively a pair of gill-clefts, which have coalesced and come to open medianly. He afterwards showed[2] that it arises in Teleostei as two lateral depressions just like gill-clefts. In the preceding pages I showed that in Elasmobranchs there was a primitive branchial sense organ over the angle of the mouth, and with this sense organ an associated ganglion, the Gasserian, and also, that just as in the nerves of other gill-clefts a supra-branchial nerve was afterwards developed from this ganglion in connection with the extension of the branchial sense organs of the mouth cleft. I need hardly say that I see in these facts a strong additional support for Dohrn's view.

Segmentation of the Head.

Admittedly this is one of the most difficult problems in Vertebrate morphology, and I cannot flatter myself that I am nearer a solution of it than other zoologists. But it may be remarked that the tendency of recent researches has been to increase the number of segments recognisable in the Vertebrate head. In ordinary sharks with five true gill-clefts, Marshall and Van Wijhe recognised nine segments, but Van Wijhe rejected Marshall's olfactory segment, and Marshall did not regard the hyoid as composed of two segments. I should increase the number to at least eleven in sharks with four roots to the vagus, and apparently Dohrn would agree with this number, but his segments might not be quite the same.

Indeed, at present it is impossible to solve the problem with any degree of probability, and it is a question whether it ever will be solved. Hence the following table is only a tenta-

[1] Dohrn, 'Ursprung der Wirbelthiere.'

[2] Dohrn, "Studien, &c.," 'Mittheil. a. d. Zool. Station zu Neapel,' Bd. iii I. "Der Mund der Knochenfische."

tive one, and is only meant to give a general view of the results of the researches recorded here. In passing I may remark that Dohrn's recent criticism of Ahlborn's[1] paper on this point seems to me to meet the objectionable points so successfully that further criticism is unnecessary.

In connection with this table the reader would do well to consult the three diagrammatic figures (figs. 43, 46, 51). The same results are there shown. Fig. 43 is a diagrammatic horizontal section through the various sense organs and ganglia, and with fig. 45, which is a side view of the same structures, shows the primitive condition. Fig. 45 shows the primitive position of these sense organs over the gill-clefts. In it for simplicity, the post-branchial nerves are left out. But in fig. 46 these and the præ-branchial nerves are shown. The closed gill-clefts are also given with the absorbed branches in dotted lines. Finally, fig. 51 is meant to show the adult condition of the supra-branchial nerves, which are very diagrammatically given in fig. 46.

The Relations of the Branchial Sense Organs to the "Seitenorgane" of Capitellidæ.

Eisig[2] first suggested that these two sets of organs were homologous. Since then no one has added anything to the grounds for this homology furnished by Eisig. Until now it may truly be said that we knew nothing of the morphology of these branchial sense organs of Vertebrates. Now we do know a little, and this appears to me to place the homology of the "Seitenorgane" of Capitellids with the branchial sense organs in a very doubtful light. We have seen that primitively these branchial sense organs are not found in all segments of the body but are limited to the head, that they have special ganglia, and are special sense organs of the gill-clefts.

[1] Ahlborn, "Ueber die Segmentation des Wirbelthier Körpers," 'Zeit. für wiss. Zool.,' Bd. xl, p. 309.

[2] Eisig, "Die Seitenorgane u. beckerförmigen Organen der Capitelliden," 'Mittheil. a. d. Zool. Station zu Neapel,' Bd. i.

Segment.	Dorsal Nerve-roots.	Cleft.	Nature of Sense Organ of Cleft.	Ganglion.	Supra-branchial Nerve.	Head Cavity.	Ventral Nerve-root.
I . .	Olfactory	None	Olfactory organ	Olfactory	None	None	None.
II . .	Radix longa of ciliary ganglion	None, or hypophysis	Branchial	Ciliary	Ophthalmicus profund.	First	Motoroculi.
III . .	Trigeminus	Mouth	Branchial	Gasserian	Ophthalmicus superficialis less portio facialis	Second	Trochlear.
IV . .	Facialis	Absent	Branchial	Facial	Portio facialis of ophthalmicus superficialis	Third	Abducens.
V . .		Hyoid	Branchial		Ramus buccalis	?	None.
VI . .	Auditory	None	Auditory organ	Auditory	None	None	None.
VII . .	Glossopharyngeal	First branchial	Branchial	Glossopharyngeal	Supra-temporal branch	?	None.
VIII . .	Vagus I	Second branchial	Branchial	Vagus I	Supra-temporal branch	None	None.
IX . .	Vagus II, III, and IV	Third, fourth, and fifth branchials	Branchial	Vagus II, III, and IV	Lateral nerve	None	None.
X . .							
XI . .							

In all these points they differ from the Seitenorgane of the Capitellidæ and, interesting and important as Eisig's researches are, we must at present, I think, hesitate to accept the proposed homology.

Physiology of the Branchial Sense Organs.

Of this we really know nothing. Leydig, who has the honour of having first described these sense organs, thought they were organs of a sixth sense. By others they have been regarded as touch organs, and as organs for testing the water breathed. Lastly, Mayser[1] suggested that they were a low form of auditory organ, and Emery[2] instituted a comparison between the auditory labyrinth and branchial sense organs, and concludes that the two sets of organs have an analogous function. That this is the case seems now very possible; that they are concerned in the perception of wave motion is obvious enough from their structure.

I have here shown, and Professor Froriep[3] has also come to the same conclusion, that they are the special sense organs of the gill-clefts. On this view we may assume that they give notice of impending danger to the gill clefts, and so enable the latter to be closed. Of course they were existent long before an operculum was developed in any fish.

After this demonstration that these sense organs stand in some important relationship to the gill-clefts, it may reasonably be expected that experimental evidence of their real nature will shortly be forthcoming. Here a valuable field of research is open for the physiologist, and a very important one too, for researches in it may lead to a better knowledge of other Vertebrate sense organs, such as the nose and ear, which appear to have been primitively of the same nature as these branchial sense organs.

If the researches recorded here should give any impulse to

[1] Mayser, "Studien über das Gehirn der Knochenfische, 'Zeit. f. Wiss. Zool.,' vol. xxxvii, 1881.

[2] Emery, "Fierasfer," p. 48, 'Fauna and Flora of the Bay of Naples.'

[3] This was stated by Professor Froriep and myself independently.

the physiological study of these organs, they will have done a great deal. For in spite of the many brilliant researches on the structure of these branchial sense organs, which have undoubtedly told us much about their structure and distribution, we cannot till now be said to have gained a clearer insight into their true nature than we possessed after Leydig's researches. This honoured histologist and zoologist showed that they were really sense organs, but there the matter has remained for thirty-five years.

My researches on the lateral line were commenced over two years ago in Professor Semper's laboratory in Würzburg. In consequence of difficulties with the only material I then had, viz. embryos of Teleostei, they led to very little result. Afterwards they were for a time laid aside for other work. Although the results of the work in Würzburg were very barren, being made in what appeared to be a dreary and empty field, still my gratitude is none the less due, and is here expressed, to Professor Semper for his untiring advice and assistance.

To Professor Milnes Marshall, in whose laboratory the later researches on Elasmobranchs were made, my acknowledgments are due not only for the privilege of the use of his library of zoological works, but also for his valuable assistance, criticism, and advice. I also wish to express my best thanks to Professor Wiedersheim for good counsel, and to my friend Dr. L. Will, who very kindly made a number of useful extracts from Götte's great Unke work, a work which was inaccessible to me in Manchester.

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DESCRIPTION OF PLATES VIII, IX, & X,

Illustrating Dr. Beard's paper on "The System of Branchial Sense Organs and their Associated Ganglia in Ichthyopsida."

In most cases the objective and ocular used for each drawing are denoted by letters, such as Z. D, oc. 2, which signify Zeiss's objective D, ocular No. 2. The figures are mostly camera drawings, and are all reduced to one third of their apparent enlargement.

Alphabetical List of References.

I, III, V, VII, &c. Olfactory, motoroculi, trigeminal, facial, &c., nerves. *al. c.* Alimentary canal. *aud.* and *au. o.* Auditory organ. *au. gl.* Auditory ganglion. *au. n.* Auditory nerve. *br.* Brain. *br. gl.* Branchial ganglion. *br. o.* Branchial sense organ. *c. b.* Nerve connecting ciliary and Gasserian ganglia. *c. gl.* and *cil. gl.* Ciliary ganglion. *cil.* Ciliary. *cl.* Cleft. *cl.* VI. Sixth cleft. *ep.* Epiblast. *f. br.* Fore-brain. *f. gl.* Facial ganglion. *fac.* Facial. *Gass.* Gasserian. *G. gl.* Gasserian ganglion. *gl. gl.* Glossopharyngeal ganglion. *gloss.* Glossopharyngeal. *h. br.* Hind-brain. *h. c.* Head-cavity. *h.* $c._2$ Second head-cavity. *hy. cl.* Hyoid cleft. *i. e.* Indifferent epiblast. *l. l.* Lateral line. *l. n.* Lateral nerve. *l. m.* Lateral muscle plates. *m.* Mouth. *m. br.* Mid-brain. *me.* Mesoblast. *ms.* Inter-muscular septa. *n.* Notochord. *n. c. gl.* Nerve of ciliary ganglion. *n. s.* Nervous system. *olf. gl.* Olfactory ganglion. *olf. n.* Olfactory nerve. *olf. o.* Olfactory organ. *oph. pro.* Ophthalmicus profundus. *op. s.* Ophthalmicus superficialis of fifth nerve. *p. br. o.* Primitive branchial sense organ. *p. b. n.* Præbranchial nerve. *p. f.* Portio facialis of ophthalmicus superficialis—one suprabranchial nerve of facial. *p. n.* Postbranchial nerve. *p. r.* Posterior root. *r. b.* Ramus buccalis, the second suprabranchial nerve of the facial. *sb. n.* Suprabranchial nerve. *sp. c.* Spinal cord. *sp. gl.* Spinal ganglion. *sm. b.* Smell-buds. *s. t. v.* Supratemporal branch of vagus I. *s. t. g.* Supratemporal branch of glossopharyngeal. *vg. gl.* Vagus ganglion. *vg.* I. Vagus ganglion I.

PLATE VIII.

FIG. 1.—Olfactory nerve just fusing with olfactory thickening. T. ocellata. Z. D, oc. 2, camera. *olf. n.* Olfactory nerve. *olf. o.* Olfactory thickening.

FIG. 2.—Olfactory ganglion (*olf. gl.*) and olfactory thickening (*olf. o.*) fused together. Torpedo ocellata. Z. D, oc. 2, cam. luc.

FIG. 3.—Transverse section of olfactory organ (*olf. o.*) and nerve (*olf. n.*) in Rana temporaria. Z. C, oc. 2, cam. luc.

FIG. 4.—Transverse section of embryo of Rhodeus amarus, showing olfactory nerve and thickening both fused with skin. Letters as before:—*f. br.* Brain. Z. F, oc. 2, cam. luc.

FIG. 5.—Transverse section through fore-brain and olfactory organ of an embryo T. ocellata. Combined from several sections. Shows olfactory nerve and ganglion fused with thickening and connected with brain. Letters as before. Z. A, oc. 2, cam. luc.

FIG. 6.—Somewhat horizontal section through mid-brain, showing nerve of ciliary ganglion (*n. c. gl.*) just fusing with skin. T. ocellata. Z. F, oc. 2, cam. luc.

FIG. 7.—Low power view of same section. Z. C, oc. 2, cam. luc.

FIGS. 8 and 9.—High and low power drawings respectively of a somewhat horizontal section through fore and hind-brain. Shows ciliary ganglion rudiment (*c. gl.*) and its primitive branchial sense organ (*p. br. o.*). The ganglion is in course of formation from the epiblast. *f. br.* Fore-brain. *h. br.* Hind-brain. Torpedo ocellata. Z. D and A, oc. 2, cam. luc.

FIG. 10.—Horizontal section through a young Torpedo embryo, showing ciliary ganglion still fused with its sensory thickening. Also shows motor-oculi nerve (III). *c. gl.* Ciliary ganglion. *p. br. o.* Primitive branchial sense organ. III. Motoroculi. *G. gl.* Gasserian ganglion. *hy. cl.* Hyoid cleft. *m. br.* Mid-brain. *h. c.* Heady-cavity. *f. gl.* Facial ganglion. Z. A, oc. 2, cam. luc.

FIG. 11.—Drawing under high power of ciliary ganglion and its primitive sense organ of the preceding section. Late stage, but still intimate fusion with skin. Also origin of suprabranchial nerve of ciliary ganglion (opth. profund) from skin. Suprabranchial nerve (*s. br. n.*). Z. F, oc. 2, cam. luc.

FIGS. 12 and 13.—Similar drawings to Figs. 10 and 11 respectively. Letters as before. *gl. gl.* Glossopharyngeal ganglion.

FIG. 14.—Horizontal section through mid-brain and anterior portion of hind-brain. Shows course of fifth nerve, which lies just under skin, but is not yet fused with it. No ganglion yet present. T. ocellata. Z. C, oc. 2, cam. luc.

FIG. 15.—Fifth nerve fused with its sensory thickening (*p. br. s. o.*), and proliferation of Gasserian ganglion from the skin. *G. gl.* Gasserian ganglion. T. ocellata. Z. C, oc. 2, cam. luc.

FIG. 16.—Similar figure to preceding one. T. ocellata. Z. C, oc. 2, cam. luc.

FIG. 17.—Section through hind and fore-brain. Shows Gasserian ganglion just before its separation from the skin. T. ocellata. Z. A, oc. 2, cam. luc.

FIG. 18.—Small piece of a horizontal section of a Torpedo embryo. Shows hyoid præbranchial nerve (*pb. n.*) lying in epiblast and not yet separated from it. *G. gl.* Gasserian ganglion. *f. gl.* Facial ganglion.

PLATE IX.

Fig. 19.—Transverse section through hind-brain of a Torpedo embryo. Facial nerve (VII) just on point of fusion with its sensory thickening. Gill-cleft (hyoid) just about to form. Z. C, oc. 2, cam. luc.

Fig. 20.—A similar section. Facial nerve just fused with skin, and its postbranchial (*p. n.*) passing on to muscles of cleft. Z. A, oc. 2, cam. luc. A later stage of facial ganglion in Fig. 42.

Fig. 21.—Facial ganglion leaving skin, and still connected by two suprabranchial nerves (*s. b. n.* 1 and *s. b. n.* 2). Z. D, oc. 2, cam. luc.

Fig. 22.—Horizontal section of a Torpedo embryo. Facial ganglion fused with auditory, but line of demarcation is obvious. Facial has just left the skin, and is leaving a suprabranchial nerve (*s. b. n.*) behind it.

Fig. 23.—Part of a transverse section through the auditory region of a Torpedo embryo. Auditory nerve (VIII) not yet fused with auditory thickening (*au. o.*). Z. F, oc. 2, cam. luc.

Figs. 24 and 25.—Auditory just fused with auditory thickening, and ganglion proliferating. Letters as before. T. ocellata. Z. F, oc. 2, cam. luc.

Fig. 26.—Low power drawing of a horizontal section, such as the two preceding figures form part of.

Fig. 27.—Transverse section, rather oblique, through hind-brain of a frog embryo. Shows auditory nerve and thickening on one side, and vagus nerve and ganglion on the other. *au. n.* Auditory nerve. *vg.* Vagus nerve. *vg. gl.* Vagus ganglion. Z. A, oc. 2, cam. luc.

Fig. 28.—High power drawing of auditory portion of preceding. Shows auditory nerve not yet separated from skin. Z. F, oc. 2, cam. luc.

Fig. 29.—Transverse section through auditory region of an Elasmobranch embryo. Shows auditory ganglion and auditory thickening intimately fused together. Auditory involution as yet only partial.

Fig. 30.—Similar section under low power. Auditory involution complete.

Fig. 31.—Highly magnified drawing of auditory portion of last section. Shows intimate fusion of ganglion and thickening, and proliferation of cells of thickening into ganglion. Many nuclear figures near proliferating portion.

Fig. 32.—Transverse section through hind-brain of a Torpedo embryo. Shows glossopharyngeal nerve (IX) just fused with its primitive branchial sense organ (*p. br. o.*). Z. C, oc. 2, cam. luc.

Fig. 33.—Similar section to preceding. Vagus nerve (X) just before fusion. Z. C, oc. 2, cam.

Fig. 34.—Similar section. Vagus nerve just fused with its thickening. Postbranchial branch (*p. n.*) passing on to muscles of cleft. Z. D, oc. 2, cam.

Figs. 35 and 36.—Portions of similar sections to preceding. Portions of

vagus ganglion (*vg. gl.*) above gill-cleft, and just separating from skin. In separating, leaving a nerve behind. *p. br. o.* Branchial sensory thickening. *phr.* Pharynx. *cl.* Cleft. T. ocellata. Z. D, oc. 2, cam. luc.

PLATE X.

Fig. 37.—Horizontal section through head of a Torpedo embryo. Shows hyoid præbranchial nerve (*p. br. n.*) forming in epiblast.

Fig. 38.—High power view of small piece of preceding section, showing hyoid præbranchial nerve (*p. br. n.*) in epiblast. Z. F, oc. 2, cam.

Fig. 39.—Horizontal section through Torpedo embryo. Vagus ganglion separating from the skin. Lateral line (*l. l.*) growing backwards and pushing indifferent epiblast (*i. e.*) away. *sp. gl.* Spinal ganglion. Z. A, oc. 2, cam. luc.

Fig. 40.—High power view of part of preceding section, showing lateral line forming (*l. l.*) indifferent epiblast (*i. e.*) being pushed away, and lateral nerve (*l. n.*) splitting off from thickening. *me.* Mesoblast. T. ocellata. Camera luc.

Fig. 41.—Later stage of lateral line. Further back in trunk. High power camera lucida. Shows same things as preceding drawing. T. ocellata.

Fig. 42.—Drawing combined under camera from several horizontal sections of an Elasmobranch embryo. Shows various cranial ganglia fused with their branchial sense organs. *p. br. o.* Primitive branchial sense organ. *m. br.* Mid-brain. *c. gl.* Ciliary ganglion. *G. gl.* Gasserian ganglion. *f. gl.* Facial ganglion. *au. gl.* Auditory ganglion. *gl. gl.* Glossopharyngeal ganglion. *vg. gl.* I. First vagus ganglion. *vg. gl. c.* Second, third, and fourth vagus ganglion. *l. l.* Lateral line. *l. n.* Lateral nerve. *au.* Ear. *n.* Notochord. *sp. c.* Spinal cord.

Fig. 43.—Diagrammatic horizontal section through the various branchial sense organs and their ganglia. The reader should conclude nothing from the cerebral vesicles figured here. There is probably at least one between the trigeminal and seventh nerves, and it is not figured here.

Fig. 44.—Part of a horizontal section of a six weeks' old salmon. Shows the position and segmental arrangement of the branchial sense organs (*br. o.*) in the trunk. *in. s.* Intramuscular septa. *n.* Notochord. *me.* Mesoblast.

Fig. 45.—Diagram of lateral view of an Elasmobranch embryo. Shows the central nervous system as plate not yet involuted, the posterior roots of the cranial nerves (*p. r.*), the branchial sense organs, the dorsal eye (*oc.*), mouth, and gill-clefts. Letters as before.

Fig. 46.—Similar diagram to show the branches of nerves to gill-clefts. The aborted branches in dotted lines. Also shows formation and direction of various suprabranchial nerves (*s. b. n.*). Vagus represented as supplying

in all five clefts. This figure is a more diagrammatic view of Fig. 51, which represents nature more or less accurately.

Fig. 47.—Horizontal section of a Torpedo embryo, showing rudiment (*cl.* VI) of a sixth true branchial cleft.

Fig. 48.—Low power drawing of transverse section through nose of an adult Triton, showing Blaue's smell-buds (*sm. b.*).

Fig. 49.—High power drawing of two such smell-buds. Z. F, oc. 2, cam. luc.

Fig. 50.—Diagrammatic transverse section through the gill-bearing region of an Elasmobranch or other Ichthyopsid. Nervous system not yet closed in. On the left side the gill muscle plate is shown, and on the right the gill-cleft. *h. c.* Head-cavity. *n. s.* Nervous system. *p. r.* Posterior root. *n.* Notochord. *p. br. o.* Branchial sense organ. *br. gl.* Branchial ganglion. *l. m.* Lateral muscle plate. *p. n.* Postbranchial nerve. *al. c.* Alimentary canal.

Fig. 51.—Diagram taken partly from my own drawings and partly from Dr. Marshall's. Shows the ganglia and various branches of the cranial nerves. Also mouth (*m.*) and gill-clefts ($cl._1$, $cl._2$), &c. For lettering see general list.

The Development of the Mole (Talpa Europea), the Ovarian Ovum, and Segmentation of the Ovum.

By

Walter Heape, M.A.,
Demonstrator of Animal Morphology in the University of Cambridge.

With Plate XI.

The Ripe Ovarian Ovum.

The position of the ripe ovarian ovum in the ovary is betrayed by the rounded semi-transparent Graafian follicle in which it lies, projecting prominently on the surface of the ovary.

If an ovary containing such a follicle be held firmly with a pair of forceps on a slide, and the follicle be pricked with a needle, or better still, sharply gashed with the point of a fine scalpel, the ovum spirts out on to the slide together with a not inconsiderable amount of clear transparent fluid, the liquor folliculi.

In accordance with the degree of ripeness of the ovum thus obtained it is more or less completely invested by a mass of epithelial cells, in the midst of which it lay in the discus proligerus within the follicle.

These epithelial cells are radially arranged round the ovum (fig. 1). The cells of the innermost layer are more or less elongated and their inner end, tapering somewhat, rests upon a thick transparent membrane which surrounds the ovum, the so-called zona radiata (the zona pellucida of the older observers).

The shape of the cells of this inner layer varies according to age, as van Beneden has observed (No. 4), but they invariably have the aspect of an epithelial investment. To this layer of cells the misleading term of membrana granulosa has been applied.

The Zona Radiata.

The zona radiata in fully ripe ova (*vide* figs. 1 and 2) is a clear transparent membrane with a granular outer border upon which the surrounding cells of the discus proligerus rest (fig. 1).

The inner portion of this membrane is so transparent that the outlines of the epithelial cells may clearly be seen through it.

The origin of the granular outer portion has not been satisfactorily traced; it may possibly, according to Balfour (No. 1), be due to the presence of the remains of the primary vitelline membrane, within which the zona radiata has been subsequently produced. On the other hand, the appearance may be due to the irregularity of the surface of the zona radiata itself, this latter circumstance being in its turn occasioned partially by the close adhesion of the surrounding cells of the discus (fig. 6), partially by the open mouths of numerous canals which pass radially through it, and to which I shall call attention directly (fig. 7)).

I have not myself attempted in this paper to trace the development of the ovarian ovum or its membranes, and must therefore at present leave this question without further discussion.

The thickness of the zona varies in the two specimens represented (figs. 1 and 2) between ·008 and ·011 mm. The two ova themselves were both completely surrounded by the cells of the discus proligerus, but in the one drawn in fig. 2 the greater portion of these cells has been carefully detached.

The radially striated appearance of the zona has long been shown to be due to a vast number of fine canals passing radially through it. These canals I find open on the inner side of the

zona by a slightly dilated mouth, while on the outer side of the zona they communicate with the exterior by a considerably wider opening (fig. 7). Into the external openings of these canals I have been able to trace prolongations of those cells of the discus which are immediately in contact therewith (fig. 7), and there appears to me no room for doubt that the contents of these follicular cells are thus rendered available for the nutriment and growth of the ovum.

Owing to the extreme minuteness of the canals it is quite possible that they are only rendered visible by the protoplasm of the follicular cells, which is less transparent than the zona itself, passing through them, and the fact that careful observers have not succeeded in detecting these pores would be accounted for by the cessation of the nutrient process at the time of observation. I may add I have observed the radial canals through the zona in optical sections of various whole ova, as well as in many actual sections of ova situated within the Graafian follicle.

I have before mentioned that the close investment of the ovum with follicular epithelium cells is in accordance with the degree of ripeness of the ovum itself. When the latter is fully mature only a very small number of, and in some instances no, epithelium cells are carried out with it upon the rupture of the follicle. Thus the attachment of the epithelium to the zona ceases when the ovum becomes mature, and no further nutriment is required, and this is of itself some further proof of the nutrient function of the follicular epithelium cells.

I myself never detected any follicular cells within the zona, such as has been described by Lindgren (No. 15), von Sehlen (No. 21), and Virchow (No. 22); nor have I seen any trace of a micropyle in the zona, such as M. Barry (No. 3) and others held to exist.

The Vitelline Membrane.

Within the zona radiata and enclosing the ovum itself in all those ripe ovarian ova examined by me, is a second very thin

membrane, the vitelline membrane (*vide* Reichert No. 18, Meyer No. 17, and van Beneden No. 4). In the ovum drawn in fig. 1, this membrane may be seen where a space exists here and there between the zona and the ovum.

In fig. 2 no space was to be distinguished with the magnifying power used (Zeiss D) for the drawing, but in fig. 7, whic is a drawing of a portion of the circumference of the same ovum with a higher magnifying power (Zeiss, imm. 3), a narrow space is clearly shown between the ovum and the zona, and a very fine membrane is there discernible closely covering the ovum. This membrane is, however, most clearly visible in fig. 8, which is the drawing of an ovum in which maturation has taken place; in this specimen there is a considerable space between the vitelline membrane and the zona, the former being rendered still more evident on account of the contraction of the material of the ovum itself within the vitelline membrane. The space between the vitelline membrane and the zona radiata I propose to call the circum-vitelline space.

The development of the membranes, about which there has been considerable discussion, I propose to consider in a future paper.

The Yolk.

The ripe ovarian ovum itself is composed of food-yolk of two kinds—(1) homogeneous, partially transparent, vesicular bodies, (2) minute highly refractive granules of various sizes,—and of a network of protoplasm which divides the yolk into rounded or cubical masses such as I have endeavoured to represent in figs. 2 and 7. The two kinds of yolk are similar to those described by most of the observers of Mammalian ovarian ova. It is worthy of remark, however, that I found no globules in the Mole's ovum similar to those described by Beneden and Julin (No. 6), and figured by those authors in their paper (No. 7) on the ova of Cheiroptera.

The difference in the density of the yolk in various Mammalian ova is very remarkable and would, I suspect, if examined

with regard to the early phases of development, throw some light upon the curious differences which then occur.

Kolliker (No. 14, 2nd edit., p. 44) and Schulin (No. 20), declare that the human ovum is markedly deficient in yolk vesicles when compared with the ovum of the Cat or the Cow. Bischoff (Nos. 8, 9, 10, 11), in his figures of the ova of the Rabbit, Dog, Guinea-Pig and Deer, shows that the Deer's ovum is not filled with such a dense mass of yolk as is that either of the Dog or Rabbit, while the ovum of the Guinea-Pig is remarkably transparent, a statement in the latter case with which Reichert's (No. 18) and my own observations fully coincide (*vide* fig. 21). The Mole's ovum must be classed in this particular with that of the Rabbit and Dog, while the Bat's ovum it appears is similar to that of the Guinea-Pig.

The network, which has as far as I know hitherto only been observed in Mammalian ova by Schäfer (No. 19) in young ovarian ova of the Rabbit, was very distinct in the ovum represented in fig. 2. A similar appearance was noted in other ova, but in a considerable number no such network could be detected. There appears to me, however, good reason to believe that the appearance is due to a protoplasmic reticulum in the meshes of which the food material lies.

The Nucleus.

In all those ova in which the nucleus was observed it was placed excentrically; the density of the yolk being so great it could not be distinguished when lying in the centre of the ovum. It was found to be either circular or oval in optical section, and bounded by a distinct membrane. In the ovum represented in fig. 2, the nucleus is indicated by a circular ring; its contents could not, however, be observed owing to the density of the supervening yolk, the network before spoken of being seen overlying the nucleus.

In figs. 3, 4, 5, I have drawn the nuclei of three ova which I obtained from the female from which the ovum drawn in

fig. 2 was taken. I tore open these ova and isolated their nuclei; the one represented in fig. 3 was flattened by withdrawing the fluid in which it was immersed from beneath the coverslip, the other two are, as nearly as may be, not under the influence of pressure. In all of them a homogeneous nuclear substance bounds a central clear space in which lies the nucleolus. Besides the nucleolus a small number of large and small highly refractile irregular-shaped bodies are contained within the nucleus.

In fig. 4 the nucleolus, which is not bounded by a membrane, consists mainly of an aggregated mass of minute granules, a single larger granule being embedded in the midst of these. A ring of four very large, irregular granules surrounds the nucleolus and a few fine granules are contained in the peripheral nuclear substance.

In fig. 5 the boundary of the nucleolus is more distinct, and the transparent space surrounding it is well marked. A few small and medium-sized granules are contained within the nucleolus, while a number of small particles are suspended in the nuclear substance.

Fig. 3 shows still further differentiation. The nucleolus is free from granules, is contained within a definite sharply-marked outline, and within the nucleolus itself an appearance of radial striation may be noticed. A ring of large granules (broken by pressure) surrounds the nucleolus and a number of smaller particles are distributed peripherally.

It appears therefore, from an examination of these three nuclei, that a single nucleolus only is present, and that a variable number of larger or smaller or of both-sized granules are also contained within the nucleus. The nucleolus is situated in a transparent central portion of the nucleus, while in the peripheral homogeneous nuclear substance a number of minute highly refractile granules are suspended. A few larger irregular-shaped granules may be arranged close around but distinct from the nucleolus, while the latter may itself contain smaller granules. Whether or not the isolated granules are to be regarded as nucleolar material is a question I do not pretend

to decide, but the appearance of the nucleoli in figs. 4 and 5, considered in connection with the researches of Grüber (No. 12) on the nuclei of Protozoa, would suggest that such is the case.

Mature Ovarian Ovum.

The phenomena of the maturation of the ovum I have not had an opportunity of observing in all its phases, but I have been fortunate enough to obtain a fully mature ovarian ovum (or one almost in a mature condition) which has been represented in fig. 8.

In this latter the ovum lies freely within the zona radiata and is separated from it by a considerable space, the circum-vitelline space in which, according to v. Beneden, is a fluid, the circum-vitelline fluid. The vitelline membrane is here distinctly seen on account of the contraction of the substance of the vitellus.

The ovum itself is very dense and contains a number of dark granules not observed in less mature ova; it is separated from the vitelline membrane by a narrow space excepting (1) at certain points where pseudopodia-like processes of the vitellus project across the space and are attached to the vitelline membrane, and (2) at one spot where no contraction of the ovum has occurred. At this latter point the vitellus is more transparent than elsewhere, and the nucleus may there be seen in close approximation to a dark oval body lying immediately outside the vitelline membrane, while a second more transparent oval body in which is a central dark mass may be seen lying in the midst of the circum-vitelline space. These two bodies are the polar bodies (*p. b.*), the second of which has but just been produced; while the nucleus seen within the ovum is the female pronucleus (*f. p.*).

It is possible to describe the vitellus as composed of a cortical more clear, and a medullary granular portion such as Beneden (No. 5) describes in the mature ovarian ovum of the Rabbit, but the boundary of these layers is by no means easy to define. The light-coloured space in which the nucleus is

situated is continuous undoubtedly with the cortical portion (*vide* Beneden, loc. cit.).

When fully mature the vitellus again swells out and there is no space seen between the ovum and the vitelline membrane. At the same time the distinction between cortical and medullary portions ceases to be visible, and the female pronucleus probably retires to the centre of the ovum, judging from its behaviour in other types, and is no longer to be seen owing to the density of the yolk. In this condition the ovum is fully ripe and is ejected, by the bursting of the follicle, into the funnel-shaped opening of the Fallopian tube.

Beneden (No. 5) describing the process of the formation of polar bodies in the Rabbit's ovarian ovum, concludes that the germinal vesicle is ejected to form those bodies, and that the ovum becomes therefore a non-nucleated cell, while Balfour (No. 2, vol. i, p. 61) in criticising this statement suggests that further observations "will demonstrate that part of the germinal vesicle remains in the ovum to form the female pronucleus."

The latter supposition, I would venture to think, is justified by the observations above recorded, and I would suggest that it is possible the supposed "Monerula" condition of the ovum described by van Beneden was due to the fact that the opacity of the ovum and the retirement of the nucleus to its central portion at the time the observation was made, prevented it from being seen.

Impregnation.

Impregnation takes place in the upper portion of the Fallopian tube.

In fig. 10 an ovum is represented which was obtained from the upper end of the oviduct; it has not yet divided into segments, but spermatozoa have found their way within the zona radiata and two nuclei (the male and female pronuclei) may be seen approaching one another.

The vitellus is irregularly granular (for the sake of clearness this condition has not been represented in the figure) and is

closely surrounded by the vitelline membrane. The circum-vitelline space is narrow, and within this space a number of spermatozoa and also two polar bodies were observed. The ovum appears to have expanded considerably since the maturation stage when the circum-vitelline space was wide, for in the ovum represented in the figure the polar bodies are greater in diameter than is this space, and thus cause a depression on the surface of the ovum.

As to the number of spermatozoa which actually enter the substance of the ovum I have no more evidence than appears in the drawing (fig. 10), in which if my interpretations are correct, a single male pronucleus is present. No movement was observed among the spermatozoa within the peri-vitelline space; they appear to be attached there, and indeed in the case of a similarly-conditioned ovum when the zona was removed, these spermatozoa remained fixed to the vitellus and were not pulled away with the zona.

I have always failed to observe either the presence of cilia or a rotation of the ovum within the zona such as Bischoff describes.

The Segmentation.

The first segmentation furrow gives rise to two oval segments of which one is generally somewhat larger than the other, although the difference in size may be quite inconsiderable, or there may be no difference at all, as is practically the case in the ovum figured (fig. 11), the one segment being 20·25 × 15·5, the other 19·75 × 16.

The vitellus in both segments is finely granular and presents no difference in character in either segment.

The nuclei are distinct, numerous spermatozoa are contained within the circum-vitelline space, and two polar bodies are visible.

The zona radiata, with its rough granular outer border, is distinctly striated.

The measurements of the segments of several other ova of this stage are given in the table on p. 169.

Four segments now make their appearance by the division of the first two (fig. 12). Each of the segments is of different size, and indeed in every ovum which I have examined of this stage with one exception, such is the case. (For measurements *vide* table p. 169.)

Spermatozoa and polar bodies are still to be seen in the circum-vitelline space and have been found in ova as old as fifteen segments, although the former in fewer numbers and both considerably shrunk.

From this point the segmentation continues entirely irregularly, and the segments formed are of various sizes. Figs. 13 to 19 are sketches of ova with six, seven, eight, nine, seventeen, and larger numbers of segments. A table of the measurements of the segments of several of them will be found on p. 169.

Throughout I have been unable to discover that the segments are arranged in any definite manner, and have not found it possible to distinguish the slightest difference in the contents or in the density of any segments during the process of segmentation. In size the segments also appear to me to bear no relation the one to the other.

Segmentation is carried on during the passage of the ovum down the Fallopian tube, and is completed by the time the former reaches the uterus.

After the close of segmentation, and when the ovum has descended into the uterus, but not until then, the segments are clearly divided into two layers. The arrangement is as follows:—A single layer of cubical hyaline segments completely surrounds, except at one point, an inner mass of rounded or polygonal densely granular segments. The gap in the outer layer of hyaline segments is filled up by one of the granular segments (fig. 20). The cause of this sudden change is not absolutely clear, but I would suggest the following hypothesis as a probable explanation.

I have little hesitation in stating that not only have the outer layer of segments become more hyaline than heretofore, but the segments of the inner mass have become denser, and

contain larger granules and more granules than they hitherto have done; and I would suggest that the yolk material originally contained in all the segments alike, has been transmitted from those occupying the outermost layer to those lying within, in order to allow the former segments to perform the function, and exhibit such activity as is now required of them.

In order to make my meaning clear I will briefly state what these changes are; for a detailed account of this subject, however, I must refer the reader to a former paper (No. 13). Very shortly after the segmented ovum enters the uterus it dilates into a vesicle—the "blastodermic vesicle." In the early stages of this formation the change is due entirely to the activity of the outer layer of segments; first by a flattening out, and secondly by the multiplication of these cells; the inner mass meanwhile remaining passively attached to one point on the circumference of the vesicle.

Later the cells of the inner mass assist in the formation of the vesical wall, and eventually the whole of the inner mass, with the exception of a very small number of cells which form hypoblast, become so disposed. The outer layer of segments and the largest portion of the inner mass of segments, therefore, together form the epiblast of the blastodermic vesicle.

Eventually the epiblast of the embryo is formed from a portion of the wall of the vesicle, the hypoblast of the embryo from a small number of the inner mass-segments, while the mesoblast has its origin from both epiblast and hypoblast layers.

Primarily, therefore, the blastodermic vesicle is formed by the energy of the outer layer of segments, and I would suggest that the differentiation of the outer and inner segments, the one from the other, after the ovum enters the uterus, is due to the transmission of yolk contained in the outer segments to the inner segments, this transmission being performed in order that the changes about to take place in the constitution of the ovum may more readily be performed.

Van Beneden, in his description of the Rabbit's ovum in 1875 (No. 5) describes the first two segments formed as, the

one larger and hyaline, the other smaller and containing a more dense vitelline material. The hyaline segment he calls the epiblastic, the more opaque segment the hypoblastic sphere. He then describes the order of the subsequent segmentation phenomena, and declares that the segments derived from the primary hyaline epiblastic sphere gradually grow round those formed from the primary hypoblastic sphere, and there results a structure precisely similar to that described above (p. 166), which he calls the "metagastrula" stage. This metagastrula Beneden compares with the gastrula of lower types, and he derives the epiblast of the blastodermic vesicle and of the embryo from the outer "epiblastic" spheres, and the hypoblast and a portion of the mesoblast from the inner "hypoblastic spheres."

There can be little doubt however, that Beneden's account of the derivation of the layers is incorrect, and that the greater portion of the inner segments, as well as the whole of the outer segments, give rise to epiblast. When this is considered, and when the probable homologies of the primitive streak are recollected, any comparison of the so-called "metagastrula" of the Mammalian ovum with the gastrula of lower types is found to be impossible, and the significance of whatever differences may exist in the two primary segments is rendered unimportant.

In the absence of any figures in Beneden's paper I have been unable to compare the appearance of the segments he describes in the Rabbit's ovum with those I have examined in the Mole, but I have myself examined segmenting ova of the Rabbit, and have isolated the segments the one from the other, in order the more clearly to compare them, and in no case have I been able to distinguish the slightest difference in the density or constitution of these segments.

If my observations are correct, then, the differentiation of the segmentation spheres into two layers in the fully segmented ovum is not a primary differentiation such as Beneden discerns, but a secondary differentiation due to the peculiar circumstances of nutrition and development attending the formation of the Mammalian embryo.

	1 Segment.	2 Segment.	3 Segment.	4 Segment.	5 Segment.	6 Segment.	7 Segment.	8 Segment.	9 Segment.	10 Segment.
Ovum with 2 segmts.	18·5 × 15	18 × 14								
" 2 "	20 × 16	18·5 × 15								
" 2 "	20·25 × 15·5	19·75 × 16								
" 2 "	19·25 × 14	19·5 × 17·25								
" 4 "	16	13·5	14·5	15						
" 4 "	15	16 × 14·5	13 × 11·25	15·25 × 13·5						
" 4 "	14·5	14·25 × 13·75	16 × 13	15 × 13·5						
" 4 "	9·5	9	9	9						
" 5 "	9	9	9	9	9					
" 6 "	11	13·5	12 × 11	13·5	15 × 14	14				
" 7 "	15 × 14	13·5 × 11	12 × 11	12 × 11	12·5 × 11	12 × 11·5	12 × 10			
" 7 "	15 × 11	12 × 11	12 × 9	14 × 12	11 × 11	12 × 11	12 × 11			
" 7 "	13 × 11	15 × 14	13 × 11	12 × 11	13 × 10	12 × 10	14 × 13			
" 7 "	13 × 12	12 × 11	13 × 12·5	12 × 11	12·5 × 10	11·5 × 11	13 × 11			
" 7 "	15 × 14	14 × 12	13 × 13	13 × 11	12	11 × 9	13			
" 7 "	14 × 11	13 × 12	12·5 × 11	12	12 × 12	12	13			
" 8 "	11 × 10	12·5 × 10	12·5 × 10	12 × 12	13 × 11	12	10 × 10	?		
" 9 "	11	10	12·5	10	13·5	11	10	11	?	
" 10 "	10 × 9	9 × 9	9 × 8	9	8	9	8	8	8	9

Summary.

The membranes surrounding the ripe ovarian ovum are two: (1) a single outer, thick, zona radiata, with a granular peripheral and a transparent inner portion, pierced radially by fine canals through which nutriment is obtained by the ovum from the follicular cells (of the discus proligerus) immediately in contact with the zona: (2) an inner very delicate vitelline membrane which closely covers the ovum itself; and between these membranes is a space, the circum-vitelline space. The confirmation of Reichert's (No. 18), Meyer's (No. 17), and van Beneden's (No. 4) observations as to the presence of the inner delicate vitelline membrane appears of some interest as many embryologists are still sceptical of its existence, while the relation of the follicular cells with the radial canals of the zona supports the view as to the source of the nutriment of the ovarian ovum. On the other hand the fact that nothing was seen comparable to a micropyle in the zona, such as M. Barry (No. 3), and Meissner (No. 16), described, nor any follicular cells within the zona such as Lindgren (No. 15), von Sehlen (No. 21), and Virchow (No. 22), have observed, is some further proof that the conditions of the material investigated by these authors was abnormal.

The yolk contained within the ovum, which is of two kinds: viz. (1) homogeneous vesicular bodies, (2) minute highly refractile granules, is contained within the meshes of a protoplasmic reticulum; it is dense and contains no large globules such as Beneden (Nos. 6 and 7) describes in theBat's ova. The rounded or oval nucleus contains a single centrally placed nucleolus and a variable number of smaller or larger granules, which may possibly be considered as nucleolar material.

During maturation the vitellus becomes divided into a medullary granular, and a cortical non-granular portion, the circum-vitelline space between the zona radiata and the vitelline membrane is enlarged, while the vitellus itself contracts away from the vitelline membrane excepting (1) here and there where pseudopodia-like processes connect the two, and (2) at

one spot where the polar bodies are formed. At this latter place two polar bodies may be seen in the specimen figured, outside the vitelline membrane, whilst the nucleus remains as the female pronucleus lying in the peripheral portion of the ovum. Finally, the vitellus again expands and the nucleus retires to the centre of the ovum and is no longer to be seen. Assuming that these observations are correct, Beneden's description of the ejection of the vesicle to form the polar bodies and the subsequent non-nucleated condition of the ovum must be considered erroneous.

Impregnation appears to be effected by a single spermatazoon, although a considerable number of spermatazoa find their way through the zona and may be seen lying passively in the circum-vitelline space.

The segmentation occurs while the ovum travels down the Fallopian tube. Two and then four segments are formed, after which the course of segmentation is irregular. The segments themselves are of irregular size and do not appear to be divisible into two kinds (epiblastic and hypoblastic) as Beneden describes. After its entrance into the uterus, a division of the segments into an outer hyaline layer and inner deeply granular mass takes place, and I would suggest the hypothesis that the vitelline matter which was originally contained in all segments alike has been transmitted from the outer segments to the segments lying in the interior of the ovum, in order that the former segments may the more readily and actively multiply and flatten out to form the wall of the blastodermic vesicle. The epiblast of the vesicle and of the embryo is derived from the whole of the outer layer and by far the largest proportion of the inner mass of segments. The hypoblast is derived from the small remaining portion of the inner mass and the mesoblast, subsequently, from both epiblast and hypoblast layers. This being the case, the division of the segmentation spheres, by Beneden, into epiblast and hypoblast spheres from the time when the first two segments were formed, is incorrect; and at the same time the theory of a comparison of the metagastrula stage with the gastrula of other animals is likewise untenable.

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EXPLANATION OF PLATE XI,

Illustrating Mr. W. Heape's Paper on "The Development of the Mole (Talpa Europea), the Ovarian Ovum, and Segmentation of the Ovum."

Reference Letters.

c. v. s. Circum-vitelline space. *f. e.* Follicular epithelium. *f. p.* Female pronucleus. *g.* Granules within nucleus. *m. c.* Mucous coat. *m. p.* Male pronucleus. *n.* Nucleus. *nc.* Nucleolus. *p. b.* Polar body. *r. c.* Radial canals. *sp.* Spermatozoa. *v. m.* Vitelline membrane. *y.* Yolk. *z.* Zona radiata.

All the figures are drawings of the ova of the mole, except Fig. 21, which represents a guinea-pig's ovum. Figs. 13—19 have been copied for me by Mr. H. A. Chapman.

Fig. 1.—Ovarian ovum not yet ripe, surrounded by follicular epithelial cells, *f. e.* The outline of these cells is to be seen through the transparent zona, *z.* The outer edge of the zona is granular. A vitelline membrane may be distinguished here and there. (Zeiss D, occ. 2.)

Fig. 2.—A ripe ovarian ovum. A few follicular epithelial cells only remain attached to the zona. Network of protoplasm permeating the vitellus. (Zeiss D, occ. 2.)

Figs. 3, 4, and 5.—Nuclei of three mature ovarian ova, similar to that drawn in Fig. 2. Single nucleolus, *nc.*, and large and small granules, *g.*, in each nucleus. Yolk vesicles, *y.*, and granules surrounding nucleus in Fig. 4. (Zeiss F, occ. 2.)

Fig. 6.—A portion of the circumference of ovum represented in Fig. 2,

showing the uneven surface of the zona, *z.*, and its granular outer border. The radial canals, *r. c.*, passing through the zona, and the circum-vitelline space between the vitellus and the zona, *c. v. s.* (Zeiss, imm. No. 2.)

Fig. 7.—Small portion of the zona of the same ovum, more highly magnified. The follicular epithelial cells, *f. e.*, are here seen to be prolonged into processes which enter the radial canals, *r. c.*, passing through the zona. The vitelline membrane, *v. m.*, surrounding the ovum is here shown. (Zeiss, imm. No. 3.)

Fig. 8.—Mature ovarian ovum. Vitellus has contracted, and a large circum-vitelline space, *c. v. s.*, left between vitelline membrane, *v. m.*, and zona, *z.* Vitellus has also contracted within the vitelline membrane, excepting where amœboid-like processes connect the two, and at a spot where a polar body, *p. b.*, is seen lying against but outside the vitelline membrane. A second polar body lies freely in the circum-vitelline space. The female pronucleus, *f. p.*, is present within the ovum. (Zeiss D, occ. 2.)

Fig. 9.—More highly magnified portion of the same ovum, showing two polar bodies, *p. b.*, outside, and female pronucleus, *f. p.*, within the vitelline membrane.

Fig. 10.—Impregnated ovum. Male and female pronuclei, *m. p.* and *f p.*, are visible within the ovum. Two polar bodies and numerous spermatozoa, *sp.*, in the circum-vitelline space. (Zeiss D, occ. 2.)

Fig. 11.—Ovum segmented into two.

Fig. 12.—Ovum segmented into four.

Fig. 13.—Ovum segmented into six.

Fig. 14.—Ovum segmented into seven.

Fig. 15.—Ovum segmented into eight.

Fig. 16.—Ovum segmented into nine.

Fig. 17.—Ovum segmented into fifteen.

Figs. 18 and 19.—Ova segmented into a number of segments.

The ova represented in Figs. 10—19 were all obtained from the Fallopian tubes of moles.

Fig. 20.—Fully segmented ovum obtained from the anterior end of the uterus of a mole. The segments are now divided into an outer layer of hyaline segments, *o. l.*, and an inner mass of densely granular segments, *i. m.* There is one spot on the circumference of the ovum where the hyaline segments are not continuous, and here one of the granular segments is interposed. The layer of hyaline material *m. c.*, outside the zona, is a coating of mucous material which has collected there since the ovum entered the uterus.

Fig. 21.—The ovum of a guinea-pig, segmented into four to show the large yolk granules and the transparent appearance of the segments.

The Development of the Cape Species of Peripatus.

By

Adam Sedgwick, M.A.,
Fellow of Trinity College, Cambridge.

PART II.

With Plates XII, XIII, and XIV.

The Segmentation of the Ovum and Formation of the Layers.

Unsegmented Uterine Ova.

The ovum is composed of a spongework (Pl. XIII, fig. 13), the strands of which consist of an apparently hyaline and structureless material and contain a small number of highly refractile globules of various, but always small, size. Globules of a similar nature are also found in the spaces of the spongework.

In the ovum of Peripatus Balfouri, and to a much smaller extent in the ovum of P. capensis, a number of angular bodies, staining slightly deeper than the rest of the reticulum, and in unstained specimens having a somewhat yellow tint, are present (Pl. XIII, fig. 19, *s.b.*). At first I took these structures for a kind of yolk material contained in the meshes of the spongework, but a more careful examination has led me to believe that they are merely nodal expansions of the latter; they undoubtedly present the appearance of being continued at their angles into the strands of the reticulum (Pl. XII, fig. 1, *s.b.*). The property which they possess of

staining more deeply than the rest of the reticulum—a property which is only visible in sections through the ovum—is probably merely apparent and due to the fact of their greater mass.

There is then no yolk material in the ovum, unless these bodies and the small highly refractile globules which are present in very small numbers are to be regarded as such.

The sponge-like structure of the ovum of P. capensis is very conspicuous. The meshes of the spongework must be occupied in life by a structureless fluid, for they contain in preserved specimens nothing presenting any structure, excepting the small number of globules and granules already mentioned. It can hardly be doubted, when the large size of the egg is considered, that some not very remote ancestors of the Cape species must have possessed an ovum, heavily charged with food yolk. We may further conclude from what we know of the relationship of the food yolk to the protoplasmic reticulum in other eggs, that this yolk must have been contained in the meshes of the reticulum, which now contain only fluid. This view is strongly confirmed by the fact that in a species of Peripatus, living at the present day and closely resembling Capensis, viz. P. novæ zealandiæ, the ovum is considerably larger than that of Capensis (1·5 × 1 mm.), and contains a large amount of food yolk. Our knowledge of the structure and early development of the ovum of this species is very small. It has been described by Hutton (6) and Kennel (8), and I have cursorily examined ova removed from hardened specimens. But the latter were too ill preserved to enable me to arrive at any satisfactory conclusions as to their structure and early development. There can, however, be no doubt on the following points:—(1) They are very large, (2) they have a thick chitinous shell, and (3) they are very heavily charged with food yolk. The shell of the Cape species, is as already stated, a somewhat delicate, transparent, structureless, but dense membrane, and within it, and much more closely applied to the ovum, there is a second, apparently similar, but more delicate membrane.

It is interesting to notice here the small size (·04 mm.) of the ovum of the West Indian species as described by Kennel. The eggs of these three species seem to form a perfect series in regard to size[1] and amount of yolk, and it would be extremely interesting to compare their structure and the early stage of their development. I regret, however, the materials for this comparison are to a great extent wanting; for, although we know more of the development of the West Indian species than of the New Zealand one, thanks to the researches of Kennel, still, as I have pointed out in my former paper (this Journal, vol. xxv), the latter are too incomplete to permit of any profitable comparison.

After this account of the general structure of the ovum of the Cape species, I will describe the special features of the unsegmented uterine ovum at its different stages.

The unsegmented ova, which I have found, seem to belong to two distinct stages, each of which presents special features. There are (1) the stages before the conjugation of the male and female pronuclei; (2) the stages after that event.

1. The ova of this stage all belong to P. Balfouri; they are distinguished externally by the small size of the dark area in the living ovum (*vide* Part 1, Pl. XXXI, fig. 2), and by the apparent absence, in surface views, of the polar bodies. All of them, at least all those of which I succeeded in preparing good sections, presented indications, more or less distinct, of a male pronucleus, and in all polar bodies were being formed.

The ovum contained an irregular central cavity which, however, was not so well marked as in later ova. The reticulum was slightly denser round the nucleus than elsewhere. This slight increase in density is the cause of the small opaque spot in the fresh ovum. The nucleus was placed in the middle of the long axis of the ovum near the surface, and presented a different structure in every ovum of this stage which I examined.

[1] Greatest length of ovum of P. novæ zealandiæ, 1·5 mm.; of P. capensis, ·5—·6 mm.; of P. Balfouri, ·4—·5 mm.; of P. Edwardsii, ·04 mm. All the known species of Peripatus are viviparous and bring forth fully developed young.

In all, except one which I have figured (Pl. XII, fig. 1), it appeared to be undergoing changes in connection with the formation of the two polar bodies. I have four ova of this stage, and they all presented structures which I take to be the male pronucleus.

The polar bodies are two in number; when fully formed they have a diameter of about ·016 mm. Each of them contains a small number of deeply-staining bodies which are placed close together in the centre and represent the nucleus (Pl. XII, fig. 1). I have never seen an ordinary vesicular nucleus in a polar body.

The male pronucleus varied in the different ova. It was always placed near the surface almost opposite the female nucleus.

2. The ovum in which the male and female pronuclei had united[1] all presented essentially the same features so far as the body of the ovum was concerned, but differed in the structure of their nuclei. The structure of the ovum will readily be understood after an examination of fig. 8, Pl. XII. There is a well-marked cavity traversed by irregular strands of protoplasm. The network is much closer round the nucleus than elsewhere. This feature of the perinuclear protoplasm is much more marked than in the earlier ova, and causes the large opacity noticeable in surface views of the ova of this stage (Part 1, Pl. XXXI, fig. 1).

The polar bodies present no essential differences from those of the previous stage. They persist during the early stages of segmentation.

The nucleus presented different appearances in the different specimens. In all, however, it was distinguished by its large sizes, and it seems to be the cause of the central transparency of the dark patch seen in fresh ova. Its structure will be described below.

[1] I have not observed the conjugation of these nuclei. I assume its occurrence from the analogy of other animals. In any case the ova I am about to describe were undoubtedly older than the preceding, and the nucleus is the first segmentation nucleus.

The Segmentation.

The general features of the segmentation have already been described in Part 1 (this Journal, vol. xxv).

The first furrow passes through the centre of the opaque patch, and at right angles to the long axis of the ovum. Each of the two segments resulting from this consists of a small opaque portion, which contains the nucleus and is closely applied to the opaque part of the other segments (Part 1, fig. 4). A careful examination of this ovum shows that the furrow has not completely separated the two segments from each other, but that they are connected by strands of protoplasm forming a loose network between them. This network is simply a looser part of the ordinary protoplasmic network described at the beginning of this article. There are, however, no such strands between the most superficial parts of the opaque area; in this region the furrow is for the moment complete. Soon, however, the clearer protoplasm (where the network is looser and continued into the still looser network between the two segments) extends upwards on the inside of the dark patch, so that when four segments are formed by the second vertical furrow each dark patch is surrounded on all sides by a layer of the looser reticulum (Part 1, Pl. XXXI, fig. 5), which is here as elsewhere continuous with the reticulum of the adjoining segments.

Two changes now occur: (1) the pale, clearer, and larger part of the four segments begins to break up into smaller, irregular masses of varying size, which, however, are seen on careful examination to be connected with each other by a wide-meshed reticulum, and (2) a third furrow appears dividing the four dark patches, which I have called the ectoderm cells, into eight patches or cells (Part 1, fig. 7). This furrow may be looked upon as corresponding to the horizontal furrow, which ordinarily follows the second vertical furrow in the segmentation of the ovum. The ovum therefore now consists of eight ectoderm cells, and four large and a number of smaller endoderm masses, all connected together by a wide-meshed reticulum,

and placed immediately beneath the egg-shell around a central cavity—the segmentation cavity. Each ectoderm cell presents in the fresh specimen (Part 1, fig. 7) (1) a central clear area—the expression of the nucleus; (2) around this a dark area—the expression of the dense protoplasmic reticulum around the nucleus; and (3) a paler circumferential area, which is more marked on the outer than on the inner border of the cells. This is the expression of the looser part of the reticulum, which is continuous internally with the reticulum of the adjoining cells, and externally with the clearer masses constituting the rest of the ovum, and called by me the endoderm masses (Pl. XIII, fig. 19). All the above elements are arranged round the central cavity, which was present even in the unsegmented ovum. Fig. 14, Pl. XIII, is a diagrammatic representation of a transverse section through the ectoderm cells at this stage; it shows the continuity of the looser circumferential parts of the reticulum of the two cells (the endoderm masses are not represented in this figure).

The next divisions take place parallel to the long axis of the ovum, and result in the formation of sixteen ectoderm cells arranged in four rows, each row containing four cells. A diagrammatic transverse section of such an ovum is shown in fig. 15, Pl. XIII, in which the endoderm masses are represented. This section also shows the segmentation cavity around which the various elements are arranged.

The further changes which may be considered as belonging to the segmentation stages consist in the continued and regular subdivision of the ectoderm cells, and in the continued breaking up of the endoderm masses into smaller bodies. Fig 8 in Part 1 represents a fully segmented ovum. It consists of a small patch of ectoderm cells, and a number of irregular branched endoderm masses. Both the ectoderm cells and endoderm masses are placed immediately beneath the egg-membrane round the segmentation cavity. A diagrammatic representation of a transverse section of such an ovum is shown in Pl. XII, fig. 10, and Pl. XIII, fig. 17 is a drawing of an actual section through such an ovum in sitû in the uterus.

The reticulum which connects the endoderm masses is shown —highly magnified—in fig. 7, Pl. XII. It lies immediately beneath the egg-shell and consists of pale, hyaline strands, which at the nodes spread out into flat expansions. The strands contain a small number of strongly refractile globular bodies. This drawing was made from an uninjured ovum preserved in sublimate and acetic acid. The reticulum connecting the ectoderm cells is shown in Pl. XIII, fig. 12, made from an ovum of the same age and prepared in the same way as the last. This drawing represents one corner of the ectoderm patch; three whole cells and parts of three others are represented, and they are all seen to be connected by a loose reticulum. The protoplasm immediately round the nucleus has a granular appearance owing to the closeness of the reticulum. The connection between the ectoderm patch with the larger endoderm masses, as seen with a lower power, is shown in fig. 9, while fig. 6 represents two small endoderm masses connected together by, and giving off in all directions, fine strands as seen under a higher power.

The endoderm masses now begin to draw together (*vide* figs. 10—13, Part 1), and form a ring-like mass applied all round the edge of the ectoderm patch. This ring-like mass is thicker at each end of the ectoderm disc than in the centre (Part 1, fig. 12), where, indeed, it is sometimes interrupted (Part 1, fig. 13). Pl. XIII, fig. 16, represents a transverse section through the edge of an ovum at this stage.

The process of drawing together of the endoderm masses is still further continued and the ectoderm cap becomes bent round the concentrated solid mass so formed (Part 1, fig. 15). Pl. XIII, fig. 20, represents a transverse section through an ovum at a slightly later stage, in which a cavity, the future mesenteron, has begun to appear.

The ectoderm cap now gradually (Part 1, fig. 18) grows round the endoderm mass, and almost completely encloses it. The one unenclosed point persists as the blastopore (Part 1, fig. 20). While this process has been taking place the cavity in the endoderm mass has become larger, and on the completion of the process

of epibole opens to the exterior through the blastopore. The ovum has now reached the gastrula stage (*vide* Part 1, figs. 19 and 21).

Before passing on to consider the structure of the gastrula and the formation of the mesoderm, I desire to call attention to certain remarkable features in the preceding development.

1. The embryo at the gastrula stage, and in all the earlier stages of development, is a syncytium. I have already pointed out that the segmentation is not a true segmentation. The segments do not separate from one another, bnt remain connected by a loose protoplasmic network. What happens is this: the nucleus of the fertilised ovum divides and gives rise to the nuclei of the two first segments. This causes a redistribution in the arrangement of the protoplasmic network, but no break in its continuity. In the unfertilised ovum there is only one centre—the nucleus—around which the protoplasmic reticulum is especially dense; while in an ovum with two segments there are two points—the two nuclei—around which we find an especial closeness of the reticulum. In an ovum with four segments there are four points around which the reticulum presents this especial density, and so on to the close of segmentation (Part 1, figs. 1, 4, 5). In each case the centre is occupied by a nucleus derived by division from the nucleus of the fertilised ovum. But this is not all, and I come to the second remarkable feature I wish to mention.

2. No part of the nucleus or centre of force of the unsegmented ovum enters the clear endoderm masses. Its products remain confined to the ectoderm cells. The endoderm masses are, during the segmentation stages, without any structure resembling a nucleus as ordinarily described, and they do not acquire one till the disco-gastrula stage when the endoderm masses are beginning to aggregate (Pl. XIII, fig. 16.) The endodermal nuclei, when they do appear, differ considerably in structure from the nuclei of the ectoderm. They are larger and have a very irregular shape; and further, they do not present the usual karyokinetic figures so characteristic of a dividing nucleus, but divide directly.

We may therefore look upon the ovum of the Cape Peripatus as presenting two different modes of segmentation, neither of which are instances of complete cleavage in the ordinary acceptation of the term.

First, there is the segmentation preceded and apparently determined by the division of the nucleus of the fertilised ovum and its products. This process gives rise to the ectoderm cells.

Secondly, there is the division of the larger and clearer vegetative part of the ovum into the endoderm masses. This process takes place contemporaneously with the first, but apparently without being governed by the dividing nucleus of the animal or ectodermic part. At any rate no part of the latter enters the endoderm masses. It is true that the endoderm masses in the fresh state do present a central opaque portion (Part 1, fig. 8,) but I was unable by any of the staining methods I adopted (borax-carmine, hæmatoxylin) to find any trace of a structure like an ordinary nucleus in preserved specimens of the segmenting stages, though nuclei were easily visible in the endoderm of the gastrula and later stages. I did find, however, in my stained section of preserved segmenting ova, that the endoderm masses presented a central portion in which the spongework was much denser than in the peripheral parts (Pl. XIII, figs. 16, 17). But this central denser portion was entirely without the especially deeply-staining chromatin so characteristic of the ordinary nucleus. This is especially shown by fig. 16. On the other hand, there are in the strands of the network of the endoderm masses small particles of a deeply-staining matter, which are neither visible in the unsegmented ovum nor in the gastrula stages, and which are not to be distinguished from nuclear chromatin. These deeply staining bodies are found in great numbers in the endoderm masses (fig. 16), and to a very small extent in the ectoderm cells. Have these central dense portions of the endoderm masses and the scattered deeply-staining bodies any hand in giving rise to the undoubted nuclei which subsequently appear? In other words, are these structures to be looked upon as

nuclei in a condition of structure somewhat different from that usually presented by nuclei? or are the nuclei of the endoderm cells derived from the nuclei of the ectoderm by migration from the latter at the disco-gastrula stage? The continuity between the reticulum of the endoderm and ectoderm cells is retained as I have said through the disco-gastrula stage (fig. 16) to the gastrula stage (figs. 20, 24—26); indeed, in the gastrula stage it becomes, in consequence of the closer approximation of the endoderm masses to the whole inner surface of the ectoderm cap (fig. 20), still more marked. The strands of the reticulum of the ectoderm cells are continued into the strands of the ectoderm masses, and the whole ovum presents the appearance of a multi-nucleated vacuolated mass (fig. 20). It may be that some of the nuclei of the ectoderm cells pass along these continuous strands into the endoderm. But against this view are these two facts: (1) I have never seen any trace of such a migrating nucleus, and (2) the structure of the endoderm nuclei of the gastrula stage is so very unlike that of the ectoderm nuclei. Compare Pl. XIV, figs. 24—26.

Before leaving this subject, I may call attention to the small bodies present in the endoderm masses in the early gastrula stage (fig. 20). These bodies do not stain so deeply as the endodermal nuclei, which are now present in small numbers, or as the small, deeply-staining bodies seen in the sections of the disco-gastrula stage (Pl. XIII, fig. 16); but they stain more deeply than the ordinary protoplasmic reticulum. Can these bodies have anything to do with the endodermal nuclei which are now appearing?

This subject is one of extreme interest, and I shall return to a consideration of it when I have described the structure of the nucleus of the unsegmented ovum and its immediate descendants.

3. The third point of interest in the development of the gastrula is the mode of origin of the cavity of the gastrula.

The solid gastrula consists of a multi-nucleated, much-vacuolated mass of protoplasm. The gut of the gastrula arises from an enlargement and confluence of the

vacuoles in the centre of this mass. The gut of Peripatus is therefore to be looked upon as a vacuole, resembling in all essential respects the cavity in the body of a ciliated Infusorian. I refer to Pl. XIII, fig. 20, which represents a section through a gastrula in which the gut is only just appearing, and to Pl. XIV, figs. 23, 24, which represent sections through a rather later stage, in which the gastrula cavity is established. In fig. 23 especially, the gut is seen to be traversed by a protoplasmic reticulum containing a nucleus, and the blastopore itself to be partially choked up by a similar reticulum. The latter feature is also seen in fig. 24 *b*, a section of a slightly older embryo, and, indeed, is characteristic of all the later gastrula stages until the definite division of the blastopore into the primitive mouth and anus. The gut vacuole, soon after its appearance, acquires an opening to the exterior through a point on the surface where the ectodermal nuclei are and always have been absent.

The Various Forms of Nuclei in the Early Stages of Development.

I have no observations on the nucleus of the ripe ovum. The facts which I have to record on the structure of the nucleus after the entrance of the spermatozoon may be described under the following heads:

1. The nucleus of the unsegmented ovum after the conjugation of the male and female pronuclei of the ectoderm cells in the early stages of segmentation.

2. The nucleus of the ovum before this event, but after the entrance of the spermatozoon.

3. The nucleus of the ectoderm during the segmentation and gastrula stages.

4. The endodermal nuclei.

1. The nucleus of the completely fertilized ovum and its immediate descendants is so large and favorable for study that I have decided to describe it first. It varies considerably in shape and structure in different ova. These variations no

doubt represent different phases in the life-history of the nucleus. It has been impossible for me with the small number (ten) of unsegmented ova at my disposal to determine their sequence. I have, however, seen it in four conditions, which differ from one another sufficiently to merit a special description; three of these were found before the beginning of segmentation, and one in an ovum of two segments.

a. A spherical structure (diameter, 0·04 mm.) bounded by a membrane, which is slightly indented at one point, where it sends in a prolongation of itself, which passes through the nucleus to become continuous with the membrane of the opposite side (Pl. XII, fig. 8). The prolongation of the membrane across the nucleus is also connected with the membrane at another point (on the lower side of the figure), and, in addition, sends off processes which ramify in the substance of the nucleus. The nucleus is made up of a fine spongework of very pale fibrils, which are continuous with the nuclear membrane and with the septum and its processes just mentioned. In this spongework are a number of deeply-staining more or less spherical bodies.

The membrane, septum, and its processes stain about as deeply as the strands of the extra-nuclear reticulum, and they appear to be continuous with the fine, pale, little staining strands, which constitute the main mass of the nuclear spongework. The pale spongework further possesses, as I have already said, a number of bodies—some elongated and branched, others globular—which are, I think, stained rather more deeply than the membrane and its offshoots, and which are likewise continued into the strands of the pale nuclear network. This latter fact is quite easy to see in the elongated branched staining fibrils, and the deeply-staining globular bodies, when carefully examined with a high power, present in many cases an angular appearance, the angles being continued into the pale reticulum.

As already stated, the nuclear membrane and septum appear precisely similar in structure to the strands of the external protoplasmic reticulum, and the latter are continued

directly into the former. The pale nuclear reticulum is also similar to the extra-nuclear reticulum, differing only in intensity of staining.

It is also directly continued into the nuclear membrane and septum. The apparently isolated, deeply-staining bodies, both globular and branched, are also, as I have said, continuous with the pale reticulum; so that this nucleus may be described as consisting of a portion of the spongework of which the ovum is composed, the nuclear protoplasm differing only from the external protoplasm in the fact that the staining matter is aggregated into special parts of the spongework instead of being uniformly diffused throughout the latter as in the extra-nuclear protoplasm. The apparent nuclear membrane is simply part of the protoplasm at the junction of the modified (nuclear) and unmodified (cell-substance) part of the protoplasmic network.

The question now presents itself; why do parts of the nuclear spongework appear more deeply stained than the rest? Either the parts thus staining are of greater mass than the rest, extending through the whole thickness of the section, while the pale strands are so fine that several of them, separated by the spaces of the meshwork, lie above one another in one transverse section; or there is a special chromatic substance, distributed at intervals in the intra-nuclear spongework. If the former is the correct answer the difference in colour between the pale and stained parts of the network is of the same nature as the difference in the colour of blood or another coloured fluid when viewed in a thick or in a thin layer.

Though there may be something in this way of looking at the deeply staining parts of the nuclear spongework, I do not think that it entirely explains the matter.

It may here be mentioned that the meshes of the extra-nuclear reticulum immediately around the nucleus are much smaller than in parts remote from the nucleus, so that in a transverse section several strands will lie one above the other in even the thinnest section, while away from the nucleus, where the meshes are coarser, a smaller number of strands will

coincide. Hence the protoplasm immediately around the nucleus appear more deeply stained than do the peripheral portions.

b. A form closely resembling the above, except in the fact that the nuclear spongework is stained slightly, though not quite so deeply as, some of the extra-nuclear protoplasm (Pl. XII, fig. 2). There are only three (in the whole nucleus) small deeply-staining masses, which are not so conspicuous as in the first form, but are more deeply stained than the membrane and septa.

Using the second of the two above-mentioned alternatives, we may state the difference between these two nuclei thus: in the first form the chromatin of the nucleus is aggregated into a number of small masses, while in the second form the chromatin is, for the most part, diffused throughout the nuclear reticulum. The word chromatin being used to denote the property which enables the protoplasm to take up and retain the staining matter. The extra-nuclear protoplasmic threads possess this property, and may be said to possess chromatin, but it is in a diffused form, as in the second form of nucleus.

c. In the third form (Pl. XII, fig. 3) the nucleus is divided by a number of septa, radiating from its centre, into chambers. The chambers are partially divided up into secondary chambers by prolongations of the septa. The septa are continuous externally with the extra-nuclear protoplasmic reticulum. It is impossible to speak of a distinct boundary of the nucleus in this form, and the substance of the nuclear septa and their prolongations is exactly similar in appearance and staining properties to the strands of the surrounding protoplasmic network or spongework.

A number of chromatin masses occur in each chamber of this radiate nucleus—they appear to lie in the offshoots of the septa into the chambers and in delicate expansions of these. But it is impossible to determine exactly the relation of these chromatin globules to the protoplasmic network in the nucleus.

This form of nucleus is most interesting, because were it not

for the chromatin masses the nucleus would be quite undistinguishable from the surrounding protoplasm, except, perhaps, by the fact that the meshes of the network (i. e. network as seen in section) are rather larger than in the protoplasm immediately around the nucleus.

The most important, and at the same time most certain, of these observations on the nucleus of the fertilised ovum of Peripatus, is that the intra-nuclear and extra-nuclear reticulum are both continuous with the so-called nuclear membrane. This continuity between the extra-nuclear and nuclear spongework is rendered still more obvious by a consideration of the next form.

d. The last form I have to describe under this head is the spindle form (Pl. XII, fig. 11). It was met with in an ovum of two segments.

The spindle is of enormous size (distance between the poles 0·06 mm.). The protoplasmic fibres composing it are absolutely the same in appearance as the rest of the cell protoplasm, and must have been largely derived from the latter. The chromatin is present in a very condensed form (i. e. deeply staining) as a number of bent rods at the equator of the spindle. Around the poles of the spindle the protoplasmic reticulum is arranged in a radiate fashion. The spindle appears not to be composed of simple fibres running from pole to pole, but of the ordinary reticulum, the meshes of which are very much elongated in a direction parallel to the long axis of the spindle. The same may be said of the fibres radiating from the poles of the spindle.

The facts which are most clearly brought out by the above observations, and about which I have no doubt, are—

1. The continuity of the nuclear reticulum with the extra-nuclear reticulum.

2. The similarity in structure between, and the continuity of, the so-called fibres of the spindle in form *d* with the surrounding reticulum; and the conclusion I have drawn from my observations is that the nucleus of the fertilised ovum of Peripatus differs from the cell protoplasm only in the manner in which the so-

called chromatin contained in the protoplasmic meshwork (both of nucleus and rest of ovum) behaves. In the nucleus it varies from a state of diffusion through the reticulum to a state in which it is condensed into the chromatin masses of forms *a*, *c*, and *d*.

In the subsequent stages of segmentation the nucleus gradually becomes smaller until at the close of segmentation it has an oval form with a long diameter of 0·016 mm. It now presents the features described by Flemming and other observers in the nuclei of the salamander and other animals.

During segmentation the nucleus generally has the third form above described: I have never seen it in a spherical, and only once in a spindle form. I conclude that these forms if they occur are very rapidly passed through.

2. **The Female and Male Pronuclei.**—I include under this head the nucleus of the ovum after the formation of the first polar body. I have no observation on the nucleus of the uterine ovum before this event.

a. Two Ova of Peripatus Balfouri with one polar body completely formed and no trace of the second.—In one the nucleus of the ovum had the spindle form and the two equatorial rows of chromatin bodies had already slightly separated from one another. It was placed near and with its long axis parallel to the surface of the ovum. The area of dense protoplasm in which it was placed was considerably smaller than in later ova which possessed the first segmentation nucleus. The spindle had a length from pole to pole of ·017 mm. It presented precisely the same features of structure as the larger spindle described above.

In the other ovum the nucleus had the form of a number of closely aggregated masses of chromatin occupying an area of ·0084 mm. The protoplasm in which they were contained did not appear to differ in any way from the rest of the denser protoplasm of the animal pole.

Male Pronucleus.—On the opposite side of the ovum, and nearly in the same transverse plane, was a small bold mass of chromatin having a diameter of ·0042 mm. It was contained

in a very small area of protoplasm in which the network was dense as at the opposite pole. This I take to be the male pronucleus.

Finally, this ovum possessed the peculiarity of presenting in surface views (Part 1, fig. 3) a number of opaque patches. These in section are seen to be due to a number of peripherally placed areas in which the protoplasmic reticulum was dense as it is around the female nucleus. The protoplasmic reticulum of these denser areas was arranged in a radiating manner around a central point; it presented no deeply-staining masses of chromatin.

b. Two Ova in which the second polar body was being formed.—In both of these the nucleus of the ovum had already divided into the definite female pronucleus and the nucleus of the second polar body, and in both the latter was attached by a wide base to the ovum. In one, however, this division has only just occurred, and the female pronucleus was in the form of some small deeply-staining masses placed close to the surface of the egg; the denser protoplasmic reticulum of the animal pole around them not being apparently modified. The male pronucleus presented the same features as in the last described ovum.

In the other ovum the female pronucleus (Pl. XII, fig. 1) was in a very different condition to the above. The chromatin masses had acquired a definite relation to the protoplasmic reticulum, and the whole structure resembled in all its essential features the chambered nucleus of the fertilised ovum (see above, p. 188). Its greatest diameter was ·029 mm. At the opposite side of the ovum and not quite in the same plane (though for the sake of convenience the two structures are combined in one figure), there was a large (·025 × ·016 mm.) reticulated structure, which I take to be the male pronucleus (Pl. XII, fig. 1). This male pronucleus was much nearer the centre of the egg than those previously described, as though it were in the act of moving to the female nucleus. The network in this nucleus was of varying degrees of fineness, and was more deeply stained in some

parts than in others; the main strands were obviously continuous with the surrounding membrane, which in its turn was obviously continuous with the very loose reticulum outside.

I have no observations on the transformation of the simple male pronucleus of the early stages into this complicated structure, nor have I any on the transformation, quite as remarkable, of the few chromatin masses which represented the female pronucleus in the last described ovum into the complicated structure present in this case.

3. The Nucleus of the Ectoderm in the gastrula and later stages.—I have already (p. 190) said all that I at present have to say about this nucleus. It is much smaller than the earlier nuclei, and not specially favorable for study. I have little doubt, however, that the network of which it is composed is continuous with the external spongework.

4. The Endodermal Nuclei.—As I have already said there are apparently no nuclei in the endoderm masses of the segmenting ovum, or, in other words, no part of the first segmentation nucleus enters, so far as I could see, these masses during the segmentation. At any rate there can be, I think, but little doubt on one point, viz. that the endoderm masses do not during the segmentation contain any structure like a nucleus as ordinarily described. They do contain, as I have already said, a densely reticulated central area, but this is without any deeply-staining chromatin so characteristic of a nucleus. Can this area represent a nucleus, perform the functions of a nucleus for these endodermal masses?

Without venturing to decide the question I may draw attention to two facts brought out by the study of the large nuclei described under heading 1 (p. 185). These are: (1) The nuclear spongework is perfectly continuous with the extranuclear spongework, and (2) the amount of concentrated deeply-staining matter may be very small, as in the undoubted nucleus of fig. 2 in which the three masses in the figure represented the whole of the especially deeply-staining matter present.

The question, therefore, presents itself; what is the essential

part of the nucleus? Is it the spongework or is it the deeply-staining parts of the spongework? A comparison of figs. 2 and 3, in which the amount of deeply-staining matter is so different, favours the first view, viz. that the essential part of the nucleus is the spongework; while on the other hand the facts about the male and female pronuclei described on p. 190 are in favour of the second view, viz. that the deeply-staining matter is the all important part of the nucleus. For in these cases we have a stage in which the nucleus is represented only by a mass of deeply-staining matter, which subsequently enters into a more complicated relation with the surrounding reticulum in order to give rise to the vesicular form of nucleus ordinarily found.

It is, therefore, impossible to decide which, if either, of these two views is correct. Indeed, it seems useless to discuss the matter except in connection with the functions of the nucleus. The nucleus appears to be a kind of co-ordinating centre for a given mass of protoplasm, and as such it may be looked upon as a centre from which force emanates. If this is so, need it have any essential structure beyond being the point to which all the strings of the protoplasmic spongework converge—in other words, such a structure as that possessed by the two poles of the spindle in Pl. XII, fig. 11? Is it not conceivable that a centre of this kind is necessary to the well-being of all masses of protoplasm beyond a certain size; and that if they do not derive such a centre from a pre-existing centre they acquire one *de novo*? May not the complexity of structure which the nucleus ordinarily presents be a secondary feature, and indicative of a higher organization of the protoplasmic mass containing it? Or, to put the matter in another way, is the complicated structure of the nucleus as ordinarily seen the cause or the result of the peculiar properties of the nucleus?

Without venturing to put forward any hypothesis on this difficult and obscure matter, I may draw attention to a fact which favours the view that the nucleus of any protoplasmic mass is primarily a central and complicated nodal point to which the strands of the spongework mainly converge, and that

the more complicated and apparently vesicular structure which it generally presents is a secondary feature. The fact I refer to is this: the first products of the division of the nucleus, i. e. the earliest stage of the two new nuclei—I mean the poles of the spindle—are simply nodal points around which the spongework is radiately arranged, and are without any of the complexity of structure which they subsequently acquire.[1]

I now pass to the structure of the undoubted endodermal nuclei which appear at the disco-gastrula stage. They are usually larger than the ectodermal nuclei (see figs. on Pl. XIV), and are sometimes very large. They are nearly always of an angular shape, and sometimes they are branched. They consist of a fine network, which stains, and the strands of which at certain points are thickened and give rise to nucleolar-like bodies. The strands of the network are continuous with the membrane, which is itself continuous with the strands of the extra-nuclear reticulum. There is no increase in the density of the extra-nuclear reticulum round the nucleus, in fact, rather the opposite. These endodermal nuclei appear to divide directly, and they never present the figures so characteristic of the indirect division. I have figured on Pl. XII, figs. 4 and 5, some peculiar endodermal nuclei found in a young hollow gastrula. Fig. 4 differs from the ordinary endodermal nuclei in the great development of its branching processes, which appear to be continued into the strands of the extra-nuclear reticulum, and in the fact that two of them are connected by processes. Fig. 5 is peculiar for the large size, number, and peripheral arrangement of the larger staining-bodies.

The Structure of the Gastrula.

The fully developed gastrula is, as I have already mentioned, a syncytium. Its cavity is a vacuole derived by the enlargement of one or the fusion of several of the vacuoles of the

[1] For an account of observations on the supposed spontaneous origin of nuclei during development, I may refer to Balfour, 'Comp. Embryology,' vol. i (2nd ed., p. 108). The ova in all the cases there cited are large-yolked and meroblastic.

mass of endoderm. The whole embryo at this stage (Pl. XIV, figs. 24 *a*—*d*) is vacuolated, the ectoderm as well as the endoderm, but the vacuoles of the endoderm are the largest. There is generally a special layer of vacuoles beneath the ectodermal nuclei, between which strands of protoplasm pass from the ectodermal to the endodermal reticulum.

The blastopore is a slightly elongated structure (Part 1, figs. 19, 21), and is itself traversed by a loose protoplasmic reticulum (Pl. XIV, fig. 24 *b*). The endodermal layer lining the gut sends out a few processes into the gut which anastomose with the blastopore reticulum. The gut of young gastrulæ contains a largely developed reticulum (Pl. XIV, fig. 23), the remains of the previous stage. In older gastrulæ there may sometimes be seen apparently isolated masses of protoplasm (Pl. XIV, fig. 24 *a*), which, however, are probably connected with the endodermal lining and eventually drawn into the latter.

Just in front of the blastopore there is a large number of nuclei in the middle ventral line (Pl. XIV, fig. 24 *a*).

Behind the blastopore there is a special area of ectoderm in the middle line which I have called the polar area, and which possesses the following characteristics: Close behind (Pl. XIV, fig. 24 *c*) the surface is flat and, if anything, marked by a slight groove, the nuclei are more columnar than elsewhere, and there is a larger quantity of protoplasm outside the nuclei than in most other parts of the ectoderm. Further back (Pl. XIV, fig. 24 *d*) there is in the middle line a fairly large area of protoplasm containing one or more large round nuclei.

The polar area extends from the blastopore backwards for a distance in this embryo (figured in Part 1, fig. 21) of about ·07 mm. The nuclei in this area will give rise to the nuclei of the primitive streak.

The protoplasm of the polar area is vacuolated in the ordinary way. Fig. 21, Pl. XIII, represents a drawing under a higher magnifying power of the hinder part of the polar area of this stage.

Figs. 22 *a—c*, represent a series similar to the above through the polar area of a rather older embryo. The front part of the polar area has a well-marked groove (Pl. XIII, fig. 22 *a*) which is the primitive groove.

FORMATION OF THE MESODERM.

The nuclei of the mesoderm are derived from the nuclei of the polar area. The latter increase largely in number (Pl. XIV, fig. 25 *b*) and form a primitive streak. An early stage of this process is shown in figs. 22 *a*, *b*. It begins at the front end of the area, but soon the nuclei of the whole area are implicated. They are constantly met with in a state of division.

In the next stage, stage A, figured in Part 1, fig. 22, a well-marked primitive streak is visible when the embryo is examined from the surface.

A series of sections through such an embryo (Pl. XIV, figs. 26 *a—d*) show that the blastopore is still traversed by a reticulum (figs. 26 *a*, *b*), and that the primitive streak is largely developed (figs. 26 *c*, *d*), and its front part traversed by a well-marked groove. In the deeper parts of the primitive streak, at about the middle of its length, there is an area of protoplasm containing two (perhaps more) nuclei, and characterised by the relative predominence of the extra-nuclear protoplasm. This area is shown in section in fig. 26 *d*. I cannot help thinking that it is derived directly from the hinder part of the polar area of the previous stage figured in Pl. XIII, fig. 21, and Pl. XIV, fig. 24 *d*. It seems to me that while the nuclei of the polar area on each side of this structure constantly undergo division (fig. 22 *c*, 24 *d*) the nuclei in this structure do not divide, but that it becomes overgrown ventrally by the proliferating lateral nuclei of the polar area (Pl. XIII, fig. 21), and thus comes to acquire a deeper position. This would seem to imply that the growth of the mesodermal nuclei in the hinder part of the polar area is a bilateral process, that the cells on each side of the middle line only proliferate; and I think that a careful examination of the anterior part of the polar area

shows that the growth of nuclei there also is a bilateral one, though the bilateral nature of the growth is not so obvious as it is behind. The reason of this is that behind there is a median structure—the hinder part of the polar area with its round nuclei—on each side of which the growth appears to take place, while in front there is no such well-marked median structure, but there is the groove; and I think that a careful examination of the relation of the growing nuclei to this groove shows the bilateral nature of the growth. I refer in support of this to figs. 22 *a*, 25 *b*, 26 *c*, which are all sections through the front part of the primitive streak, fig. 22 *a*, being of course from the youngest of the embryos; and to figs. 25 *a*, 26 *b*, which are in each case the last section through the blastopore. It is difficult to say whether 25 *a* is to be regarded as passing through the hind end of the blastopore or through the front end of the streak, and in this figure there are nuclei, which must be regarded as mesodermal, placed in a position which looks very much as though they were derived from the row of nuclei which extend between the ectodermal and endodermal nuclei.

Again, in fig. 26 *b*, we see similarly placed nuclei in the act of division, with what must be regarded as mesodermal nuclei on their inner borders.

Further back (figs. 22 *a*, 25 *b*, 26 *b*) the blastopore is represented only by the groove, and it is more difficult to satisfy oneself on the point.

However, I am inclined to think that the growth of primitive streak nuclei is a bilateral one, in the anterior as well as in the posterior part of the primitive streak, though I admit that the evidence in favour of this view is not entirely satisfactory.

If I am correct in this supposition, and in my conjecture that the primitive groove is a rudimentary posterior part of the blastopore (it is so considered in other tracheate embryos), then the development of the mesoderm in Peripatus consists in an ingrowth of mesoderm from the lips of the blastopore and resembles that described in so many other forms.

The mesodermal nuclei of the primitive streak now grow

forward in two bands—one on each side—between the ectoderm and endoderm (Pl. XIV, figs. 26 *a* and *b*, *mb.*). They seem to arrange themselves on the strands, connecting the ectodermal and endodermal reticulum, and they constitute the mesoblastic bands. A series of vacuoles are formed in these bands, around which the nuclei arrange themselves in rows, thus giving rise to the mesoblastic somites.

The further development I shall describe in Part 3 of this series.

Summary and General Conclusions.

The Segmentation is apparently complete, the ovum appearing to divide into ectoderm and endoderm cells.

The so-called endoderm cells are at first without a distinct nucleus, they do not get a nucleus until just before the gastrula stage.

All the cells of the ovum, ectodermal as well as endodermal, are connected together by a fine protoplasmic reticulum, which is placed, as are also the cells, immediately beneath the egg membrane, and therefore around a central space.

Each ectoderm cell consists of a central nucleus around which is a close protoplasmic spongework, which, at the outer parts of the so-called cell, becomes of a gradually looser nature until it runs into the spongework of the surrounding cells.

Each endoderm mass consists of a central denser spongework which gradually becomes looser towards the periphery of the mass until it is continued into a fine reticulum. The endoderm masses are far apart from each other and are connected by this reticulum.

The continuity of the various cells of the segmenting ovum is primary and not secondary, i. e. in the cleavage the segments do not completely separate from one another. But are we justified in speaking of cells at all in this case? The fully segmented ovum is a syncytium, and there are not and have not been at any stage cell limits. I think the cleavage should be rather described not as segmentation, but a multiplication of the nucleus or centre of force which

causes a corresponding readjustment in the density of the network at different parts of the ovum, but no break in continuity.

The Gastrula arises by a process of epibole and is at first solid.

The endoderm masses at first have no nuclei. Nuclei first appear in them during the progress of the epibole by which the gastrula is formed. I have not been able to determine the origin of these nuclei. They either arise de novo in the endoderm masses or migrate into the latter from the ectoderm. The protoplasmic network at the centre of each endoderm mass is denser than at the periphery, but is without the chromatin granules, so characteristic of a nucleus. But I have described a stage of the nucleus in the fertilised unsegmented ovum in which the chromatin granules are almost entirely absent, and in which the network presents no essential difference from the surrounding network. Again, another in which the nuclear network merges so gradually into the surrounding network, that it is impossible to point to any limit between them. I therefore think it quite possible that this central denser protoplasm in the endoderm masses may give rise to the nucleus which subsequently appears.

The gastrula is a syncytium; the ectodermal nuclei are arranged around the periphery of the ovum, while the endodermal nuclei are within. The latter are characterised by their angular shape, and by never presenting the karyokinetic figures characteristic of the ectodermal nuclei. The protoplasm of this syncytium is much vacuolated throughout, but the vacuoles are largest in the centre. These central vacuoles unite and give rise to the gut cavity, which opens to the exterior through a point on the surface where the ectodermal nuclei have always been absent. This opening is the blastopore. The blastopore, until quite late in development, is traversed by protoplasmic strands, which anastomose with similar strands projecting from the protoplasm lining the large central vacuole or gut.

The gut of Peripatus arises, therefore, as a vacuole in a

multinucleated mass of protoplasm, and the gastrula of Peripatus is a multinucleated mass or syncytium, with absolute continuity of the protoplasm of all parts of the ovum.

The Mesoderm.—After the definite formation of the blastopore, an area of protoplasm, placed in the ectodermal layer of the syncytium, and characterised by possessing several nuclei less densely packed together than elsewhere, is distinctly visible in the middle line of the ventral surface just behind the blastopore. This area I have called the polar area. Its nuclei undergo division and give rise to the densely packed mass of nuclei of the primitive streak. A part of it seems to persist for some time in the deeper parts of the primitive streak close to the endoderm.

The nuclei of the primitive streak migrate forwards between the ectodermal and endodermal nuclei, and take up their position in the protoplasm intervening between the latter.

These rows of nuclei are the mesodermal bands. They soon arrange themselves into groups around a central vacuole, and so give rise to the most conspicuous parts of the mesoblastic somites. I leave the ovum for the present at the commencement of the formation of the somites, merely stating that it is still a syncytium.

There are a certain number of facts in the above account which are of general interest and seem to deserve more discussion so far as their relation to processes in other forms are concerned. These are:

1. The connection between the intra- and extra-nuclear reticulum.
2. The segmentation.
3. The origin of the gut as a vacuole.
4. The syncytial nature of the embryo.
5. The origin of the mesoderm.

I propose to consider some of these points at once, and to defer the 5th, to Part 3 of this series.

A. The nucleus of the unsegmented ovum and of the early stages of segmentation of the Cape Peripatus are particularly favorable for study, because of their large size and the rapid

changes which they undergo. I have not been able to make out the sequence of these changes, but I hope with more material, which I expect to obtain this year, to be able to communicate some more facts concerning them in a future paper.

It is a disputed point as to whether or no the nuclear and extra-nuclear reticulum are continuous. Leydig (12), Stricker (16), Klein (9, 10, 11), and Heitzmann (5), hold that they are. So far as the nucleus of the early segmentation stage, and of the endoderm of Peripatus is concerned, I am able fully to confirm the views of these observers.

The general views I hold with regard to the nucleus are stated on p. 189 and I need not repeat them here. I only desire to point out that the opposite view, viz. that the nucleus is isolated, so far as continuity of protoplasm is concerned, is, from a physiological point of view, very difficult to accept; and I think that the burden of proof rests with him who maintains it.

The peculiar lobed structure (Pl. XII, fig. 3) of certain stages of the nucleus has been described before by other observers, notably by Balfour in his "Monograph on the Development of Elasmobranch Fishes," in the early stages of development.

Klein in his communication on this subject, refers (9, p. 175) to and confirms Stricker's (16) observations on the contractility of the nuclear spongework and its continuity with the extra-nuclear spongework in the colourless blood-corpuscles of the newt and frog. He further confirms Stricker's statement as to the disappearance of the cell membrane, and himself adds: "The nucleus is therefore a part of the cell substance specially differentiated by the presence of a membrane." Presumably Dr. Klein would still speak of a nucleus when the membrane is absent. I am not able to make out Klein's views with regard to this membrane. He says (11, p. 415): "In the convolution and basket of daughter nuclei the membrane is very indistinct and is also here due to the close position of the fibrils." I infer from this that he regards the nuclear membrane as a part of the general reticulum at the junction of the nuclear and extra-nuclear parts of the reticulum, which gets in certain stages of the nucleus a regular ar-

rangement. This at any rate is my view for the Peripatus nucleus.

Klein figures (10, Pl. 18) nuclei from the epidermis of the newt in a state of direct division. These figures resemble very closely some of the endodermal nuclei in the gastrula of Peripatus.

Klein is still more explicit as to the continuity of the nuclear and extra-nuclear reticulum in his second communication on this subject (11, p. 416).

Unfortunately I have not been able to see the papers of Stricker and Heitzmann.

Leydig in his latest communication (12) regards the spindle-fibres as parts of the ordinary reticulum (spongioplasma he calls it) with much elongated meshes (p. 9). He further looks upon the nuclear membrane as merely the outer portions of the nuclear network, and describes it as being porous, and takes the same view as Klein with regard to the continuity of the nuclear and intra-nuclear network.

Leydig also describes some accessory nuclei as occurring in certain cells. These are smaller than the main nucleus and stain less deeply. It is possible that they are structures of the same nature as those described in the endoderm of Peripatus on p. 184 of this memoir.

He refers, in this connection, especially to the small accessory nuclei which are found in many Protozoa, and which, according to Gruber (3) and Jickeli (7), are for the most part derived from the breaking up of the main nucleus. The particles resulting from this fragmentation of the nucleus seem eventually to come together again to form a new main nucleus. One would like to have some more details about this peculiar process in Infusoria, derived if possible from the study of sections. The term "fragmentation," which is applied to it apparently because the chromatic parts of the nucleus become separated from one another and scattered throughout the animal, seems to imply a distinct breaking up into small isolated portions. If this really happens the nucleus of Infusoria must differ from most other nuclei in which the chromatic

matter is a part of the nuclear network, which is itself continuous with the extra-nuclear network. I should be inclined to look upon the process as an increase in size or extension of the nucleus, such as seems to have been described by Stricker in certain leucocytes.

Pfitzner (14), on the other hand, strongly maintains the isolation of the nucleus during the whole of its life-history, and he recommends certain reagents to demonstrate this fact. But inasmuch as he himself admits (p. 72) that these reagents produce great changes in the nucleus, his negative conclusions cannot be regarded as having so good a basis as the positive results of Klein and Leydig, whom I can thoroughly confirm in the matter.

I may draw attention in passing to the similarity of the branched endodermal nuclei of Peripatus to the nuclei of leucocytes figured by Pfitzner (14, Pl. V, fig. 21).

I have not been able to distinguish nucleoli in the nuclei of Peripatus as distinct from the chromatic thickenings of the spongework. Flemming (1) says that nucleoli proper participate in forming the chromatic figures in cell division. Flemming in his work on the cell and cell nucleus (1) has not seen the continuity between the strings of the nuclear and intranuclear spongework. He does not deny its existence but holds that it is not proved.

Flemming makes the important statement that the first change observable in a cell whose nucleus is about to divide is in the extra-nuclear protoplasm, the fibres of which arrange themselves radially around two points on opposite sides and at the circumference of the nucleus. Contemporaneously with this the nuclear network begins to change, and almost immediately afterwards the achromatic spindle-fibres appear in the nucleus.

These facts seem to point to the conclusion that the actual centre of force, of which the nucleus is the seat, divides first and is followed by the re-arrangement of the cell and nuclear protoplasm. Flemming considers that the nuclear network consists of an achromatic substance containing granules of

chromatin which have the power of moving about in the network. These chromatic granules are fairly uniformly diffused in the resting nucleus, but in a nucleus preparing to divide they aggregate together in certain parts of the network. The parts of the network from which the chromatin has gone become inconspicuous and form the achromatic spindle-fibres, while the parts into which it has gone form the conspicuous deeply-staining rod-like fibres, so characteristic of a dividing nucleus. The achromatic fibres of the spindle which begin to appear at the first sign of the division of the nucleus are, on this view, parts of the nuclear network. With this view I entirely agree. The structure of the various phases of the nucleus of the ovum of Peripatus will bear the same explanation, allowing for this difference, viz. the amount of chromatic substance in the ovum of Peripatus is much smaller—so small, indeed, that even in the resting stage (Pl. XII, fig. 8) the chromatin is absent from the greater part of the network, which thus has the pale appearance of the achromatic fibres of Flemming, an appearance which is only found in the dividing nuclei of the salamander. The reason why achromatic fibres are so ittle marked in the resting nuclei of most animal cells is that they are masked by the large amount of chromatic substance they contain.

This view of the spindle-fibres is not at all opposed to Strasburger's contention (15, fig. 44) that part of them are derived from the extra-nuclear spongework; for the nuclear and extra-nuclear spongework are, as I have already maintained, continuous with each other; in other words, part of the same system.

I have seen nothing of any process corresponding to the splitting of the fibres; but this is not to be wondered at considering that I have only twice found the spindle stage of the nucleus.

B. It is becoming more and more clear every day that the cells composing the tissues of animals are not isolated units, but that they are connected with one another. I need only refer to the connection known to exist between connective

tissue cells, cartilage cells, epithelial cells, &c. And not only may the cells of one tissue be continuous with each other, but they may also be continuous with the cells of other tissues. For instance, I may refer to Fraipont's (2) work on the nervous system of the Archiannelida. He describes an intermuscular nervous plexus which is continuous with the muscle-cells and with the surface epithelial cells (2, Pl. 13, figs. 11, 16).

Instances of this kind might be multiplied from recorded observations, and are being multiplied day by day by histological observers to such an extent, that we are almost, if not quite, justified in regarding the body of an adult animal as a syncytium. It is true that the cells of the blood and lymph, and the *ripe* generative cells, are completely isolated. But the former, in their first stages of growth, form part of the syncytium; as in all probability do the latter also.[1]

This continuity, which for *a priori* reasons we should expect, has hitherto been regarded as a fact of little morphological importance and relegated to the category of secondary features. The ovum, it is said, segments into completely isolated cells; and the connection between these is a secondary feature acquired late in development. It has always been considered that the first stage in the evolution of the Metazoa was a colonial Protozoon, i. e. a mass of perfectly isolated unicellular organisms derived by complete division from a single cell.

Now, while I do not wish to exalt the facts of the cleavage and early development of Peripatus above recorded to a position of undue importance, or to maintain that of themselves they are sufficient to destroy this conception of the origin and structure of a Metazoon, I think I am justified in pointing out that if they are found to have a general application, our ideas on these subjects and others connected with them will have to undergo a considerable modification.

The ancestral Metazoon will no longer be looked upon as a colonial Protozoon, but rather as having the nature of a multi-

[1] I may refer in this connection to the processes of the follicular cells which perforate the zona of a mammalian ovum.

nucleated Infusorian with a mouth leading into a central vacuolated mass of protoplasm.

The continuity between the various cells of the adult—the connections between the nerves and muscles and sensory epithelial cells, receive an adequate morphological explanation; being due to a primitive continuity which has never been broken.

Herbert Spencer's view of the origin of the nervous system may perhaps not be so far from the mark as at first sight appeared. In any case the efforts to find out how the connection is established between the nervous and muscular tails of the ectoderm and endoderm of the lower animals should be transferred to the earliest phase of the embryo, i. e. to the segmentation stages.

Finally, if the protoplasm of the body is primitively a syncytium and the ovum until maturity a part of that syncytium, the separation of the generative products does not differ essentially from the internal gemmation of a Protozoon, and the inheritance by the offspring of peculiarities first appearing in the parent, though not explained, is rendered less mysterious; for the protoplasm of the whole body being continuous, change in the molecular constitution of any part of it would naturally be expected to spread, in time, through the whole mass.

In short, if these facts are generally applicable, embryonic development can no longer be looked upon as being essentially the formation by fission of a number of units from a single primitive unit, and the co-ordination and modification of these units into an harmonious whole. But it must rather be regarded as a multiplication of nuclei and specialisation of tracts and vacuoles in a continuous mass of vacuolated protoplasm.

At any rate I may safely say that, so far as the individual embryonic development of Peripatus is concerned, the connection of cell with cell is not a secondary feature acquired late in development, but is primary, dating from the very beginning of development.

Since making these observations on the syncytial nature of the cleavage and gastrula stage of Peripatus capensis, I

have examined other segmenting ova to see if the fact was one of general application, with negative results.

The cells of segmenting ova are generally so closely applied together and the protoplasmic strands so hidden by food-yolk, that it is difficult to be certain of the point either way. But with ova in which the segments are slightly separated from one another—and I believe there are such though I have never seen them—the observation ought to present no special difficulty.

Indeed it is a well-known fact that an incomplete separation of the cells is found in the early stages of the segmentation of centrolecithal eggs; but it has always been assumed that this was a temporary phase, and that the segments eventually separated. We now know, thanks to the researches of Heathcote (4), that this separation does not occur in the centrolecithal egg of the Myrapod, Julus; and it seems to me extremely probable that his results for this form will be found on careful examination applicable to other similar ova.

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DESCRIPTION OF PLATES XII, XIII, & XIV,

Illustrating Mr. Adam Sedgwick's paper on the "Development of the Cape Species of Peripatus, the Segmentation of the Ovum, and Formation of the Layers."

List of Reference Letters.

p. b.$_1$. First polar body. *p. b.*$_2$. Second polar body. *f. n.* Female pronucleus. *m. n.* Male pronucleus. *o. c.* Cavity in centre of ovum. *s. b.* More deeply-staining bodies in extra-nuclear part of ovum. *ec.* Ectoderm. *en.* Endoderm. *e. s.* Egg-shell. *n.* Network. *u. e.* Uterine epithelium. *bl.* Blastopore. *g. g.* Gut of gastrula. *p. a.* Polar area. *p. g.* Primitive groove. *p. st.* Primitive streak. *m. b.* Mesoblastic band.

Fig. 1.—Section through the fertilised ovum of Peripatus Balfouri before the conjugation of the male and female pronuclei. The female pronucleus is at the periphery of the ovum. Its reticulum is very loose. Large masses of deeply-staining matter are present. The extra-nuclear reticulum is not denser round either the male or female pronuclei than elsewhere. A large cavity is present in the centre of the ovum. The extra-nuclear reticulum is only drawn in immediately round the two nuclei. Elsewhere it is only indicated by shading. It is completely absent in the centre of the ovum. Peculiar bodies of irregular shape, staining more deeply and continuous by means of processes with the reticulum, are present. They are probably merely expansions of the strands of the reticulum. The male pronucleus is on the opposite side of the ovum to the female, but rather nearer the centre. It does not,

however, lie in the same transverse plane as the female nucleus, though very nearly so. It is formed of a network, precisely similar in character to the extra-nuclear reticulum. The membrane round the nucleus is continuous with both the extra- and intra-nuclear reticulum. Deeply-staining bodies are present in the intra-nuclear parts of the network. Near the female pronucleus is the second polar body, with a small portion of the first attached to it. Greatest diameter of female nucleus ·029 mm. The male nucleus measured ·025 × ·016 mm. Drawn with Zeiss's camera, F, oc. 2. Picric acid. *f. n.* Female pronucleus. *m. n.* Male pronucleus. *oc.* Cavity in centre of ovum. *p. b.*$_{1}$. First polar body. *p. b.*$_{2}$. Second polar body. *s. b.* More deeply-staining bodies in extra-nuclear part of ovum.

FIG. 2.—Nucleus of unsegmented ovum of Peripatus capensis in spherical stage. Network more diffusely stained than in Fig. 8. Borax carmine. Drawn to same scale as Fig. 8.

FIG. 3.—Nucleus of an ovum with two segments of Peripatus capensis. Nucleus divided up into compartments by specially well-marked portions of the nuclear network. The deeply-staining irregularly-shaped masses are almost certainly contained in the strand of the network. The nuclear network is most distinctly continuous with the extra-nuclear reticulum. Reticulum, both of nucleus and cell-substance, slightly stained. Greatest diameter ·03 mm. Borax carmine, sublimate and acetic. Zeiss's F, oc. 2, camera.

FIG. 4.—Three nuclei from endoderm of embryo of Peripatus capensis of stage of Pt. 1, Pl. XXXI, fig. 19. Zeiss's F, oc. 2, camera.

FIG. 5.—Nucleus of endoderm cell, lying in the gut of an embryo of the same stage. Zeiss's F, oc. 2, camera.

FIG. 6.—Two endoderm masses of Peripatus capensis, with their connections and processes. Surface view of sublimate preparation as seen with Zeiss's $\frac{1}{18}$th oil imm., oc. 2. Sublimate and acetic.

FIG. 7.—Surface view of a portion of the reticulum connecting the endoderm masses and ectoderm of a fully segmented ovum of Peripatus capensis, as seen with a Zeiss's $\frac{1}{18}$th oil imm. No endoderm masses shown. Sublimate preparation. Strongly refractile bodies in the strands of the network and sometimes in the meshes.

FIG. 8.—Transverse section through the fertilised ovum of Peripatus capensis, showing the nucleus in the spherical stage. The protoplasmic network around the nucleus is denser than elsewhere. A well-marked cavity in the centre of the ovum. Nuclear network for the most part unstained. Nuclear membrane and extra-nuclear reticulum stained. Diameter of nucleus ·04 mm. Borax carmine, picric acid.

FIG. 9.—Portion of edge of ectoderm of Peripatus capensis, with adjacent endoderm masses showing connection between the two. Surface view of sublimate preparation, as seen with Zeiss's C, oc. 2.

Fig. 10.—Ideal diagrammatic transverse section through the fully segmented ovum of Peripatus capensis, at about the stage figured in Pl. XXXI, fig. 8, this Journal, vol. xxv. *ec.* Ectoderm. *en.* Endoderm masses, connected by reticulum. *e. s.* Egg-shell.

Fig. 11.—Transverse section through an ovum of Peripatus capensis with two segments. The section passes through the centre of the nucleus of one segment. The nucleus has the spindle form, which immediately precedes division. The figure shows clearly the continuity and the similarity between the fibres of the spindle and the fibres of the extra-nuclear reticulum. A well-marked cavity is present in each segment. Greatest diameter of spindle ·06 mm. Drawn with Zeiss's F, oc. 2, camera. Sublimate and acetic.

Fig. 12.—Portion of edge of ectoderm of ovum of Peripatus capensis, almost at the close of segmentation, as seen with a Zeiss's water imm. 2. Sublimate preparation. The connection between the ectoderm cells is clearly shown, also between the ectoderm cells and the network connecting the endoderm masses. Nucleus of ectoderm indicated. *n.* Network. The apparent granulation of the ectoderm is caused by the fineness of the reticulum.

Fig. 13.—View of endoderm mass of an ovum of Peripatus Balfouri, as seen with a Zeiss's water imm. 2, to show the spongework of which the mass is composed.

Fig. 14.—Transverse section through an ovum of Peripatus capensis with eight ectoderm cells, to show the greater density of the network round the nucleus than at the periphery, where it is continued into the reticulum of the next cell. Endodermal masses not indicated. Zeiss's D, oc. 2, camera. Diagrammatic.

Fig. 15.—Transverse section through an ovum of Peripatus capensis with about sixteen ectoderm cells, somewhat diagrammatic. The endoderm is indicated. The section shows that the ovum is of the nature of a hollow blastosphere. Zeiss's D, oc. 2, camera.

Fig. 16.—Section through an embryo of Peripatus capensis at the stage of Pt. 1, Pl. XXXI, fig. 11. The endoderm masses contain a central denser protoplasm, and a number of darkly-staining granules. No nuclei visible in endoderm. Zeiss's D, oc. 2, camera.

Fig. 17.—Transverse section through the uterus, and contained a fully segmented ovum of Peripatus capensis (blastosphere stage). Zeiss's C, oc. 2, camera. *ec.* Ectoderm. *en.* Endoderm. *u. e.* Uterine epithelium. *e. s.* Egg-shell.

Fig. 18.—Slightly oblique section through an embryo of Peripatus capensis of the stage of Pt. 1, Pl. XXXI, fig. 15. Zeiss's C, oc. 2, camera.

Fig. 19.—Portion of ovum of Peripatus Balfouri with eight ectoderm cells, showing one of the corner ectoderm cells connected by a reticulum

with two endoderm masses. The endoderm masses contain a large number of irregularly-shaped yellowish bodies, *s. b.*; a few of the latter are present in the ectoderm. The outer parts of the ectoderm cells were much vacuolated, and gradually passed into the reticulum connecting them with the endoderm masses. The endoderm was in two main masses, and two or three smaller pieces in the network between ectoderm and endoderm.

Fig. 20.—Transverse section through an embryo of Peripatus capensis slightly older than the stage of Pt. 1, Pl. XXXI, fig. 15. The endoderm is largely vacuolated, and only a rudiment of the gut-cavity is present. Zeiss's D, oc. 2, camera.

Fig. 21.—Section behind the blastopore of same stage as Fig. 23, showing the most conspicuous part of the polar area. Zeiss's imm. 2, oc. 2, camera.

Fig. 22, *a*, *b*, *c*.—Series of sections behind the blastopore of an embryo of Peripatus capensis, slightly older than that from which series Fig. 24 were taken. Beginning of formation of primitive streak. Zeiss's D, oc. 2, camera.

a. Second or third section behind blastopore. Polar area marked by a slight groove, its nuclei beginning to increase.

b. Five sections behind blastopore. Groove absent, but increase of nuclei shown.

c. Twelve sections behind blastopore. *p. a.* Polar area.

Fig. 23.—Section through an embryo of Peripatus capensis at the gastrula stage (Pt. 1, Pl. XXXI, fig. 19). The gut-cavity is still traversed by a mass of much vacuolated endoderm. Zeiss's D, oc. 2, camera. *bl.* Blastopore. *g. g.* Gut-cavity of gastrula. *ec.* Ectoderm. *en.* Endoderm.

Fig. 24, *a*—*d*.—Series of sections through a gastrula of Peripatus capensis of stage Pt. 1, Pl. XXXI, fig. 21, before the appearance of the primitive streak. Zeiss's D, oc. 2, camera.

a. Five sections in front of blastopore, showing increase of nuclei between ectoderm and endoderm, similar to that which takes place at a later stage behind the blastopore. Endoderm cell lying loose in gut.

b. Through middle of blastopore. Blastopore traversed by strands of protoplasm.

c. Five sections behind blastopore, showing beginning of polar area in middle ventral line.

d. Five sections behind last, through centre of polar area. *bl.* Blastopore. *p. a.* Polar area.

Fig. 25, *a*, *b*.—Two sections behind blastopore of embryo of Peripatus capensis, slightly younger than Stage A (Pt. 1, Pl. XXXI, fig. 22). Early primitive streak. No mesoblastic bands. Zeiss's D, oc. 2, camera.

a. Immediately behind blastopore. *bl.* Marks position of blastopore in preceding section.

b. Nine sections behind the preceding. Large increase of nuclei in polar

area, constituting the primitive streak, which is marked by a groove. *p. g.* Primitive groove.

Fig. 26, *a—d.*—Series of transverse sections through an embryo of Stage A (Pt. 1, Pl. XXXI, fig. 22). Mesoblastic bands (*m. b.*) have begun to grow forward from front end of primitive streak. Zeiss's D, oc. 2, camera.

a. Through the posterior end of blastopore, and two sections behind the front end of the mesoblastic band.

b. Hindermost section through the blastopore.

c. Through the primitive streak three sections behind the blastopore.

d. Through the primitive streak ten sections behind the blastopore. This section shows a portion of the polar area lying unaltered in the deeper part of the primitive streak. *bl.* Blastopore. *p. g.* Primitive groove. *p. st.* Primitive streak. *m. b.* Mesoblastic band.

Studies on Earthworms.

By

William Blaxland Benham, B.Sc.,
Demonstrator in the Zoological Laboratory of University College, London.

Introduction.

In this series of papers I intend to describe a number of Earthworms from various parts of the world, which have been kindly put into my hands for the purpose by Professor Ray Lankester. These include some new genera, with interesting variations from allied forms, and several new species of Perichæta. But before describing these new forms I shall give—firstly, a condensed historical review of the various works on Earthworms, and a chronological record of the discovery of new facts about them.

Secondly, I shall enumerate and briefly describe all known Earthworms.

Thirdly, I shall take the various organs in order and point out their variations in different Earthworms, and the theories of various authors with regard to certain points.

Having done this, I shall proceed to describe the new forms that I have studied myself.

I wish to thank Professor Lankester for his kind advice and assistance in this work, which was carried on in his Laboratory at University College, London.

I shall not give an exhaustive bibliography, since that will be found in Perrier's work on Urochæta (28), but I shall in all cases give references for any facts I mention to a bibliography at the end of the third section, the figures in brackets referring to this bibliography.

I. Historical.

Amongst the earliest papers dealing with Earthworms anatomically are those of Savigny (1) in 1820, and of Dugés (2) in 1828, who describe numerous species of Lumbricus, which will be mentioned in Section II. Dugés figures the prostomium of some of these, and describes the genital organs; but his interpretation of the latter is wrong, since he has, like so many of the earlier writers, confused the seminal reservoirs and the spermathecæ, attributing each to the wrong sex. Other authors followed him, who, whilst contradicting him, were no nearer the truth. Von Siebold (3), for instance, suggested that the ovary was invaginated into the seminal reservoirs. Even till quite recently the "seminal reservoirs" were spoken of as "testes." I may at once say that I shall use the former name for the three pairs of large white organs in Lumbricus which originate in somites x and xi and spread into the neighbouring somites, and for their homologues in other genera.

The ovary was unknown till 1853, when d'Udekem (4) described it in Lumbricus agricola; whilst in 1856 Hering (5) supplemented our knowledge of the genital organs by his figure of the ovary and his description of its position on the posterior face of the septum between somites xii and xiii. He also showed that the oviduct was not in continuity with the ovary, but that the ova fell into the body cavity, and were conveyed thence to the exterior by the wide ciliated funnels of the pair of short oviducts which pass through the posterior septum of somite xiii to the exterior in xiv. Hering described the process of copulation, and thought that the spermatozoa passed from the sperm pore along a groove of the ventral surface to the spermathecæ; but Dr. Fraisse, in 1882 (6), describes the spermatophores of various spcies of Lumbricus, and shows that the spermatozoa do not pass directly into the spermathecæ, but are received in bodies secreted on somite xxvi. Previous authors had described as "testes" the large white sacs which are now known as "seminal reservoirs," but Hering, in this paper, describes and figures the true testes. Professor A. G.

Bourne was the first to figure them in their true position attached to the anterior septum of somites x and xi, as two pairs of small flat appendices. This figure and description occur in a paper by J. E. Blomfield (7), who describes the development of the spermatozoa in the reservoirs.

The sperm ducts were rightly described by Leo (8) in 1820, but Dugès (2) wrongly considered them as oviducts.

The nephridia or "segmental organs" also were erroneously interpreted by Dr. Williams in 1858 (40), being considered as respiratory organs. Their true function was first suggested by d'Udekem (9) in his description of Tubifex, whilst Gegenbaur (10) in 1853 published the well-known drawing of this organ of Lumbricus agricola, the histological structure of which was described by Claparède (11).

In regard to the classification of Earthworms, that of Claparède (12) in 1862 is usually followed. He divides the order Oligochæta into two families, Limicolæ and Terricolæ, but the characters of the latter, as opposed to the former, were derived from the genus Lumbricus only, and now, since the investigation of other genera, no longer hold true. These are his characters:

a. The possession of two ventral blood-vessels.

b. The presence of nephridia in the same somites with the sperm ducts and oviducts.

c. The position of the clitellum far behind the male pores.

d. The presence of a vascular network on the nephridia.

Now, Perrier's genus Pontodrilus (13) and Perichæta have no subneural blood-vessel [Microchæta[1] resembles these two genera in this respect], and very possibly others will also be found without this vessel.

The position of the clitellum is now known to vary; sometimes it is in front, sometimes around as well as behind the male pore.

The truly distinctive characters of the Terricolæ (or Lumbricinæ, as Perrier calls them), as opposed to the Limicolæ, are the following:

[1] Names or sentences in square brackets refer to results of my own research.

a. The presence of nephridia in the same somites with the genital ducts (except in some species of Perichæta and Pleurochæta (Megascolex), where nephridia are unknown in any somite, and in Pontodrilus, in which the nephridia are said not to commence[1] till the hinder region of the sperm duct, so that there are none in the somite carrying the oviduct).

b. The abundant vascular network on the nepridia and body wall.

c. The almost universal presence of a gizzard (Pontodrilus is again an exception).

d. The much smaller size of the ova and the compactness of the ovary.

But even these charcters may have to be altered as new forms are studied.

These Lumbricinæ Perrier divides into four groups, taking as a basis the relation of the clitellum to the male pore.

1. The Anteclitelliani (Preclitelliani), in which the male pore is far in front of the clitellum, include the genus Lumbricus, which Eisen (15) has lately subdivided into the genera Lumbricus, Allurus, Allolobophora, and Dendrobœna, as well as, doubtfully, Kinberg's (19) genera Alyattes and Eurydame, and Savigny's (1) Hypogæon. As these three latter genera are insufficiently described, it is doubtful whether the characteristics given by these authors justify the retention of their names.

Whilst this group contains only a few forms, the members of the other groups are numerous and mostly of extra-European origin.

2. The Intraclitelliani, where the male pore is situated within the limits of the clitellum, include the genera Anteus, Urochæta, Rhinodrilus, Microchæta, and perhaps Kinberg's Geogenia and Tritogenia.

3. The Postclitelliani have the male pore behind the clitellum, and include Perichæta, Acanthodrilus, Eudri-

[1] It is not improbable that examination by means of microscopic sections would result in the discovery of nephridia in some cases where Perrier has failed to see them with the naked eye.

lus, Digaster, Pontoscolex, Pontodrilus, Plutellus, Perionyx, Megascolex, and Pleurochæta.

4. The group Aclitelliani is formed for the genus Moniligaster, which has no clitellum, although the only specimen studied had its genital organs fully mature, and, indeed, more complicated than any other form.

The habitat of these forms is given later on, in Section II, where the names, &c., of all known Earthworms will be found.

II. Previously described Genera.

In this section I shall mention, in chronological order, and briefly notice, all Earthworms whose description I have been able to find. In the case of the genus Lumbricus I have placed all the species together at the end of this section. The anatomy of the genus Lumbricus is sufficiently well known through the works of d'Udekem (16), Lankester (48), Claparède (11), and others, so that I will refer only to Eisen's work (15), where he subdivides the genus into three subgenera:

1. Lumbricus, with the male pore in somite xv, and the prostomium embedded deeply in the first somite.

2. Allolobophora, with the male pore in somite xv, and the prostomium embedded less deeply in the first somite; this includes Dendrobœna, which Eisen at first separated, but now includes.

3. Allurus, with the male pore in somite XIII.

It seems to me that the character drawn from the prostomium is scarcely of generic importance, since forms otherwise similar have this difference (e.g. Lumbricus agricola and L. olidus), but the variation in the positions of the male pore is a good sub-generic character.

The earliest genus additional to Lumbricus was Hypogæon, formed by Savigny (1) in 1820, but, as in so many of these earlier genera, a very poor description is given, and only of external characters. Hypogæon has nine long setæ in each somite, one being in the dorsal mid-line; these setæ do not alternate in consecutive somites.

The clitellum occupies somites XXVII to XXXIX, and the whole worm has 106 somites. This specimen came from Buenos Ayres and elsewhere, and the genus has since been studied by d'Udekem, Grube (18), and lastly by Kinberg (19), who, in his description of two species, says nothing about the characteristic ninth seta, whilst no author has given a proper anatomical description of any species.

In 1844 Templeton (20) described a form resembling the wide-spread genus Perichæta, but differing from it in the presence of an interruption in the dorsal mid-line in the ring of setæ. He called the worm Megascolex cœruleus; he obtained it from Ceylon; its length was from 20 to 40 inches by 1 to 1½ inch broad; it contained 270 somites with a ring of 100 setæ on each. This must I think be referred to the genus Perichæta.

In 1845 Hoffmeister (22) described and figured several species of Lumbricus (see below), as well as the following forms which are European.

Phreoryctes, which is now placed amongst the Limicolæ.

Criodrilus has four rows of paired setæ, and is 8 to 12 inches long, and consists of 300 somites. The male pore is on somite XIV.

Helodrilus is 2 to 5 inches long, contains 160 somites, with setæ arranged as in the preceding. The male pore is on somite XV.

Neither of the latter has a clitellum.

In 1848 Rapp (21) described a worm under the name Lumbricus microchetus, which is probably the same as Beddard's Microchæta (33) from the Cape.

In 1851 Grube (23) described a peculiar form which he called Lumbricus multispinus; its chief characteristic is the possession of four bundles of 5 setæ in each somite; there was no trace of clitellum. The male pores are in somite XII, in a line with the most ventral group of setæ, and each carries a papilla. Its habitat is not mentioned, nor is the internal anatomy. Leon Vaillant (24) has founded a new genus for it, Echinodrilus. Judging from

the forward position of the male pore it is an Anteclitellian form.

Then followed Schmarda (25) in 1861, who described and formed the genera Pontoscolex and Perichæta. The former is from Jamaica and has seven setæ only in each somite, which alternate with those of the next somites, giving fourteen rows of setæ. In Perichæta the clitellum occupies somites XIV, XV, XVI; the female pore is single, median, and in XIV; the paired male pore is in XVIII; the setæ are numerous, and form a ring all round each somite. All the species described by Schmarda came from Ceylon.

P. brachycycla has no clitellum; is 88 mm. long, and 3 mm. broad.

P. leucocycla has a white ring round each somite (probably on this ring the setæ were placed); it contains eighty-eight somites. Length 300 mm., breadth 15 mm.

P. viridis contains 209 somites, fifty setæ to each. Length 100 mm., breadth 4 mm.

P. cingulata contains 100 somites, with forty setæ to each. Length 130 mm., and breadth 6 mm.

Besides these new genera, Schmarda describes two species of Hypogæon (Sav.), but says nothing about the ninth seta.

H. heterostichon came from Quito and Cuença, with the setæ diverging posteriorly. Its length is 220 mm., and breadth 11 mm. It has 263 somites.

H. orthostichon, from New Zealand, has the setæ parallel throughout the body, which consists of sixty somites. The clitellum is at somite XIV. The total length is 80 mm., and breadth 4 mm. The description of these two is insufficient to give any confidence in their validity.

Kinberg (19) added numerous new genera in 1866, most of which are so insufficiently described that it is impossible to retain their names.

Tritogenia is said to have no clitellum, and the male pores are between somites XVI and XVII. There are only six setæ to each somite. (Habitat not given.)

Mandane, from Montevideo and Patagonia, has the clitellum

on somites XII to XIV. There are four male pores situated in one species on somites XVI, XVIII, and in the other on somites XXI and XXIII.

Geogenia, from Natal, has the clitellum on somites IX to XVIII; the setæ alternate anteriorly. There are two ventral pits, one in XVI, the other in XVII (? male pores or copulatory pits), and the "lateral pores" (probably he refers to nephridiopores) are in a line with the dorsal setæ.

Alyattes, from Buenos Ayres, has the setæ separated posteriorly.

Eurydame, from St. Joseph, near Panama, has the anterior setæ paired, but the posterior ones are further apart.

Hegesipyle, from Natal, has all the setæ wide apart, except the ventral ones anteriorly.

Then follow five, which Perrier considers merely species of Perichæta, so that it will be best to use Kinberg's names specifically if they are to be retained.

Amyntas, from Guam, with fifty or sixty setæ per somite.

Nitocris, from Rio Janeiro, with fifty-two setæ per somite.

Pheretima, from Tahiti and California, with fifty setæ per somite.

Rhodopis, from Java, has fifty to sixty setæ per somite, has the clitellum on somites XII, XIII, and the male pores between somites XIV and XV (so that this differs from Perichætæ where the clitellum and male pore is constant).

Lompito, from Mauritius, with forty-four setæ per somite.

P. catinus, from Oahu, with forty setæ per somite.

Kinberg also describes two worms which he puts into Savigny's genus Hypogæon, but denies the existence of the characteristic dorsal seta.

H. havaicus, from Oahu, is 44 mm. long, contains 100 somites, and has the clitellum on somites XXIX and XXX.

H. atys, from Buenos Ayres, is 30 mm. long, by 4 mm. broad, and contains 140 somites.

Thus, in each of the species of "Hypogæon," in which a clitellum is mentioned, it differs in position. In the absence of any record of anatomical detail, it is impossible to tell what

genus Kinberg was dealing with, or indeed what significance is to be attributed to Savigny's Hypogæon.

In 1869 Baird (26) described a Perichæta (though he called it at first Megascolex after Templeton) which he had obtained from South Wales, whither it had apparently come with exotic plants.

Perichæta diffringens is 4 to 5 inches long, contains 104 somites, and has sixty setæ to each of them.

In 1869 Leon Vaillant (24) described two species of Perichæta, where the "prostate" is described for the first time.

One from Java, P. posthuma, is 18 cm. long, consisted of 100 somites, and has sixty-five to seventy-seven setæ in each somite. The setæ average about ·25 mm. in length. It has a pair of "copulatory" papillæ on the XVII, and a pair on the XIX somite, in line with the male pores. The prostate occupies two somites. The spermathecæ are four in number in the somites V, VI, VII, and VIII, opening in the anterior region; each consists of a bilobed sac. Vaillant denies the existence of a gizzard and of intestinal cæca, but Perrier has contradicted him as to the first point, and regards this worm as the same as the P. affinis.

The other species is P. cingulata, Sch., which came from Bourbon. Vaillant's description does not correspond in some points with his figure, and Perrier reserves the name for the species figured, whilst he calls the one described P. robusta. P. cingulata is 17·4 cm. long, consists of 114 somites, and has forty setæ in each somite, the length of the setæ being ·36 mm. There are no "copulatory" papillæ on or near the somite carrying the male pore. The prostate occupies only one somite. The spermathecæ have the same position as in P. posthuma.

Apparently Vaillant included other species under the name P. cingulata; these Perrier has named and separated from this form.

In his paper Vaillant numbers the somites differently from the way in which they are now reckoned. He regards the first setigerous somite as the somite I (instead of II). He also regards

the clitellum as occupying only one somite, which he calls XIII, instead of XIV, XV, XVI.

We now come to the most important work of late years on Earthworms, in which the first attempt is made to consider the relations of different forms from an anatomical standpoint. It is here that the only rational classification and generic grouping of Earthworms is first given. I refer to Edmond Perrier's works.

In 1872 he published his researches on various Earthworms contained in the Paris museum (14). In this paper he describes nine new genera, two new species of Lumbricus, and several new species of Perichæta.

The following are the new genera and their chief characters:

Anteus (A. gigas, from Cayenne).—Its length is 1 met. 16 cm., and breadth 3 cm. The clitellum occupies somites XV to XXIX, and is not continued across the ventral mid-line. The setæ are in four couples in each somite. The nephridiopores are in a line with the uppermost seta of the lateral couple (i.e. with the fourth seta from the ventral mid-line). No sperm ducts could be found, but the nephridia in the somites XI to XIX are short simple tubes, which Perrier considers as sperm ducts. No accessory copulatory organs nor ovaries are found. There is a single pair of spermathecæ in somite VII. The anterior septa are very strong, and cover the pharynx, gizzard, and seminal reservoirs.

Titanus (T. brasiliensis, from Brazil).—Length 1 met. 26 cm., and breadth 3 cm. The clitellum occupies somites XV to XXIII. The male pores are between somites XVIII and XIX, and no nephridiopores exist in this somite. The setæ are in four couples in each somite anteriorly, posteriorly become scattered, but do not alternate. The nephridiopores are in front of the second setæ, reckoning from the ventral mid-line, whether in couples or separate, that is to say, they are in line with the outer ventral setæ. No nephridiopores exist anterior to somite XIII. The seminal reservoirs are very long and consist of only one pair, extending from somite XII to XXV. The sperm

duct opens into an oval muscular pouch. No spermathecæ nor ovaries were found.

Rhinodrilus (R. paradoxus, from Venezuela).—Length 15 cm., breadth 3 or 4 mm. Prostomium elongated to form a proboscis 3 to 6 mm. long. The clitellum occupies somites XIX, XX, XXI. The setæ are in four couples, and are ornamented with two series of semicircular folds, with a concavity towards the free end. The nephridiopores are in a line with the lateral or outer couple of setæ (the setæ 3 and 4). The male pores are between somites XIX and XX in a transverse groove No spermathecæ were found.

Eudrilus.—The clitellum occupies somites XIII to XV, or XIII to XVIII. The setæ are in four couples in each somite. The nephridiopores are in line with the lateral couple of setæ. The male pores in line with the ventral (1 and 2) setæ in XVII. There is a curved chitinous penis in a sac (modified penial seta); a prostate is present. The female genital organs are very peculiar: the ovary is fixed to the oviduct and to the spermatheca, according to Perrier's interpretation of the parts.

He describes three species, all about the size of the common Earthworm:

Eu. decipiens, from the West Indies.

Eu. Lacazii, from Martinique.

Eu. peregrinus, from Rio Janeiro.

Acanthodrilus.—The clitellum occupies somites XIV to XVII, and completely surrounds the body. The setæ are in four couples in each somite. The nephridiopores are in line with the lateral couple of setæ (3, 4). The male pores are four in number in somites XVIII and XX in line with setæ 1 and 2; at each pore is a penis formed of a bundle of setæ in a sac. The genital organs are not altogether understood, and differ in each of the three species.

Ac. obtusus, from New Caledonia.—Length 66 cm. Penial setæ are blunt.

Ac. ungulatus, from New Caledonia.—Length 1 dcm. Penial setæ are recurved.

Ac. verticillatus, from Madagascar.—Length 350 mm.,

breadth 8 mm. Penial setæ are serrated. No clitellum was found.

Digaster, from Australia.—Only one species is described—D. lumbricoides. The clitellum occupies somites XIV, XV, XVI. The male pores are in somite XVIII. The setæ are in four couples. The nephridiopores are in line with the outer of the ventral couple of setæ. There are two gizzards; one in somite V, the second in somite VII.

Perionyx.—The only species is P. excavatus, from Cochin China. The length is 120 mm., breadth 4 mm. The clitellum occupies somites XIII, XIV, XV, XVI, XVII. The setæ are about 30 to each somite, and form a ring all round. The nepridiopores are not visible, though nephridia are present. The male pores are close together in a median ventral fossa in somite XVIII. The spermathecal pores are close to one another on the ventral surface of the anterior edge of somites VIII and IX. There are no intestinal cæca. The ovaries are not pedunculated.

Moniligaster.—A single species, M. Deshayesii, from Ceylon is described. Length 150 mm., breadth 6 mm. The clitellum is absent altogether. The setæ are in four couples in each somite. The nephridiopores are in front of the lateral couple. There are four male pores; two between somites VII and VIII in line with setæ 1 and 2, and two between somites X and XI dorsad of these setæ. There is one gizzard in somite VI, and a second extends through somites XIII to XXII constricted into four nearly equal portions. The genital organs are very complicated. The anterior and posterior seminal reservoirs differ from one another. The ovary is very exceptional in that it is a long sac, lying above, and on each side of the alimentary tract, in somites XII, XIII, XIV, XV.

Urochæta.—This is described in the same memoir as the preceding genera, and also in a separate memoir (28), where Perrier gives a very minute description of it, as well as an exhaustive bibliography of the literature of the Lumbricinæ at the end of the paper. Only one species is known—Urochæta hystrix, which has been found in Martinique, Gloria, Java,

and Brazil; showing thus a very wide distribution. Length 1 decim., breadth 3 mm., it consists of 220 somites. The anterior extremity tapers gradually, but there is no prostomium. The clitellum occupies somites XIV to XXIII. The setæ are in eight longitudinal rows anteriorly, all the setæ being equidistant; but posteriorly they alternate giving sixteen rows. The setæ themselves are notched at the extremity, which is exceptional. The nephridiopores are in a line with the third seta counting from the ventral mid-line, but do not follow this seta in its displacement in the posterior somites. The male pores are in somite XX, and the setæ of this somite are transversely ridged. The spermathecal pores are on the anterior edges of somites VII, VIII, IX, in a line with the nephridiopore. There is only a single pair of seminal reservoirs in somite XIII. In the posterior region of the body there is a pair of peculiar "pyriform sacs," of unknown significance, in each somite, opening to the exterior between the nerve cord and seta 1; the nephridia are also present in these somites.

The following are the new species of Perichæta, described by Perrier. I shall divide these into two groups according to the presence or absence of papillæ, on the somite XVIII, or on the neighbouring somites, or in relation to the spermathecæ. Each of these groups may then be subdivided according to the number of spermathecæ; and probably some further subdivision may be made in reference to the simple or complex structure of this organ. The arrangement of the species of this genus must be left till more have been studied. I have several species at present ready for description, and I will give a more detailed synopsis of the species in a future paper.

I. Perichætæ without papillæ.
  a. With one pair of spermathecæ.
  b. With more than two pairs.

II. Perichætæ with papillæ.
  a. With two pairs of spermathecæ.
  b. With more than two pairs.

I. Perichætæ without papillæ.

a. With one pair of spermathecæ.

P. quadragenaria, from the East Indies.—Length 210 mm., breadth 4 mm. The setæ are about 40 to each somite. The spermathecæ are only two in number, in the somite VIII; their aperture is in the anterior part of this somite, and each consists of a globular sac in somite VIII, and narrow coiled appendage with enlarged extremity in the somite VII.

P. elongata, from Peru.—Length 355 mm., breadth 4 mm. The number of setæ is not mentioned. The spermathecæ are only two in number; their pores are between somites IV and V, but whether they lie in the somite IV or in V is not stated. They consist each of a single sac, and have no accessory parts.

b. With more than two pairs of spermathecæ.

P. Houlleti, from Calcutta and Cochin China, is very fully described. Length 1 dcm.

There are forty-five to fifty setæ round each somite. There are three pairs of spermathecæ, lying in somites VII, VIII, and IX, and opening on the anterior edges of these somites. Each consists of three parts; a large ovoid sac, with a coiled tube opening into its duct, lying in the somites named; whilst a very much smaller sac, also opening at the same point, lies in the preceding somite in each case.

[P. cingulata, Sch. will come in here.]

II. Perichætæ with papillæ.

a. With two pairs of spermathecæ.

P. aspergillum (habitat unknown).—Length 370 mm., breadth 10 mm. There are about eighty setæ round each somite. The spermathecæ are in two pairs, in somites VIII and IX, with their pores in the anterior region. Each is a simple, large, and somewhat globular sac; but there are numerous smaller sacs round it, each having a separate pore; some lying in the same somite as the spermathecæ, others in the somite in front. So that, both in front and behind each

spermathecal pore, is a line of four or five smaller pores. The male pores are in somite XVIII, and each is situated on a papilla, which is studded by a row of smaller pores in front and behind, each pore belonging to a small sac internally.

P. robusta, from Bourbon and Manilla.—Its length is 150 to 180 mm., breadth 6 mm. There are forty-five setæ in each somite. There are two pairs of spermathecæ in somites VIII, IX, opening anteriorly. Each consists of a large, ovoid sac, into the neck of which opens the duct of a narrower sac. Just behind these is a much smaller sac opening to the exterior on a papilla just behind the pore of the spermatheca. Between the male pores are two papillæ, each with a small pore. Nephridia are present as extremely delicate tubules attached to the septa.

Perrier gives the name P. robusta to the worm described by Leon Vaillant (24), under the name P. cingulata, Sch.

b. With more than two pairs of spermathecæ.

P. affinis from Cochin China.—Length 110 mm., breadth 5 mm. The number of setæ is not mentioned. There are four pairs of spermathecæ lying in somites VI, VII, VIII and IX, opening anteriorly. These pores are quite lateral. Each consists of a large globular sac, with a smaller globular sac opening into its neck. There are no papillæ near their pores. The male pores are on papillæ, and there is in addition a pair of papillæ in somite XVII, and a pair in XIX.

Perrier considers this worm to be the same as Vaillant's P. posthuma.

(Horst's Perichæta from Java belongs to this group (31).)

In the same memoir Perrier describes two new species of Lumbricus.

L. americanus, from New York. Length 1 dcm. It seems to differ from L. agricola only in having the posterior pair of ciliated rosettes of the sperm duct rather larger than the anterior pair. Perrier mentions that the ciliated rosettes are not enclosed in the seminal reservoirs, but that, I think, merely depends on the state of maturity of the worm,

for in L. agricola when about half ripe, and with large seminal reservoirs, the rosettes are free.

The second new species is named Lumbricus victoris; it was obtained from Damietta (West Africa); its size is nearly the same as the preceding. The clitellum commences at somite XXVII, and occupies eight somites. The ovaries are in somite XIV, and there are three pairs of spermathecæ in somites IX, X, XI.

The following are the species of Lumbricus described by Hoffmeister (22) in 1842, with their various synonyms, according to d'Udekem (16), Perrier (14), and Rosa (17).

1. Lumbricus agricola, Hoffm.

Syn. L. terrestris, Lin..
L. herculeus, Dug.

Two pairs of spermathecæ, in somites IX and X; opening on the posterior edge of somite.

2. L. communis, Hoffm.

Syn. L. trapezoides, Dug.
L. caliginosus, Sav.
L. cyaneus, Sav.
L. ictericus, Sav.
Allolobophora turgida, Eisen (15), partim.
" " mucosa, Eisen (15), partim.

According to Rosa (17) the two species Allolobophora here named are partly synonymous.

Two pairs of spermathecæ, in somites X and XI; with apertures anteriorly.

3. L. rubellus, Hoffm., is adopted by the other writers. Two pairs of spermathecæ, in somites IX and X; opening posteriorly.

4. L. riparius, Hoffm.

Syn. L. chloroticus, Dug.
Allolobophora riparia, Eisen (15).
" " chlorotica, Rosa (17).

Three pairs of spermathecæ, in somites IX, X, XI; opening anteriorly.

5. L. olidus, Hoffm.
   Syn. L. fœtidus, Dug.
   Enterion rubidum, Sav.
   Allolobophora fœtida, Eisen (15).

Two pairs of spermathecæ, in somites IX, X; opening posteriorly.

6. Lumbricus stagnalis, Hoffm.?—Seven pairs of spermathecæ, in somites VI, VII, VIII, IX, X, XI, XII; opening posteriorly. The setæ of the four couples are wide apart.
   Syn. L. complanatus, Dug.
   Allolobophora complanata, Eisen.
7. L. pictus, Hoffm.
8. L. agilis, Hoffm. Male pores in somite XIII.
   Syn. L. tetraedrus, Dug.
   L. amphisbæna, Dug.
   Allurus tetraedrus, Eisen 15.

Other species mentioned by Dugés (2) that have not received synonyms so far as I am aware, and have not been recognised or further characterised since his time, are the following:

L. opimus, Sav.
L. mollis, Dug.
L. teres, Dug.
L. Blainvilleus, Dug.
L. tyrtœus, Sav.
L. festivus, Sav.
L. roseus, Sav.
L. dubius, Dug.
L. mammalis, Sav.
L. purus, Dug.
L. pygmæus, Sav. } Setæ separated, male pore in so-
L. vetædrus, Sav. } mite XV.
L. phosphoreus, Dug. Setæ separated, male pore in somite XIII.

As the external points only are mentioned, it may probably turn out that some of these are known under other names. They seem all to be European, as Duges does not mention, in most cases, where he obtained them.

The following are new forms of Lumbricus described by Eisen (15):

Lumbricus puter.
Syn. Allolobophora Bœckii, Rosa (17).
Dendrobœna Bœckii, Eisen (15).

L. purpureus.—Two pairs of spermathecæ, in somites IX, X; opening posteriorly.

Allolobophora subrubricunda.—One pair of spermathecæ, in somite X; opening anteriorly.

Rosa (17) has added the following European forms:

Allolobophora constricta.

All. minima.

All. transpadana.—Five pairs of spermathecæ, in somites VI, VII, VIII, IX, X; opening posteriorly.

All. profuga.—Four pairs of spermathecæ, in somites IX, X, XI, XII.

All. alpina.—Two pairs of spermathecæ, in somites IX, X.

L. meliboeus.—Two pairs of spermathecæ, in somites IX, X; opening posteriorly.

Kinberg (19) described several new species of Lumbricus, though probably some of them belong to other genera. They are extra-European:

L. Josephinæ, from St. Helena.
L. infelix, from Port Natal.
L. armatus, from Buenos Ayres.
L. Novæ-hollandiæ, from Sydney.
L. Helenæ, from St. Helena.
L. Hortensiæ, from St. Helena.
L. Vineti, from Madeira.
L. pampicola, from Montevideo.
L. tellus, from Buenos Ayres.
L. Tahitana, from Tahiti.
L. capensis, from Cape of Good Hope.
L. Apii, from California.

In 1851 Grube (23) described L. triannularis, and L. multispinus (Echinodrilus, Vaillant).

Hutton (35) has described four Earthworms which he refers to the genus Lumbricus, but they seem to belong to various genera. L. uliginosus has four male pores, two being in somite IX and two in somite X. L. campestris has a pair of male pores in somite IX.

In 1873 Perrier described a new genus (27), of which only one species is known—Plutellus heteroporus, from Pennsylvania. Length 15 cm., breadth 3 mm. The clitellum occupies somites XIV, XV, XVI, XVII, and is complete. The setæ are eight in each somite, at nearly equal distances apart, and do not alternate. The nephridiopores vary in position. The first four pores are in a line with the third seta from the ventral mid-line. The rest alternate in successive somites, one series being in line with the second seta, the other series being in line with the fourth seta. The spermathecæ are five pairs in somites V, VI, VII, VIII, IX, their pores being in the anterior region of these somites in line with the second setæ (i.e. with one set of nephridiopores). Each spermatheca consists of a sac and a short, slightly swollen blind tube opening into its neck, resembling the arrangement in some Perichætæ. The male pores are, in somite XVIII, dorsad of the first setæ counting from the ventral mid-line. There are no papillæ. The female pores are in somite X, in line with the first setæ. The seminal reservoirs are a single pair of grape-like glands in somite XII. There is a prostate and a penis in somite XVIII. The nephridia do not pass through the anterior septa, but lie wholly in one somite.

Perrier regards this genus as very likely the same as Kinberg's Hypogæon, as opposed to the similarly named genus of Savigny.

In 1874 Perrier published an abstract in the 'Comptes Rendus' (13), and in 1881 a detailed account (29) of a worm which lives on the seashore at Marseilles. He named it Pontodrilus Marionis. Its length is 80 mm., and breadth 4 mm. It consists of fifty somites. The clitellum occupies somites XIII to XVII. The setæ are rod-like. The ventral couple (1 and 2) are close together, but the other two (3 and

4) are wide apart. The nephridiopores are in a line with seta 2, but only commence in somite XIV. The male pore is in somite XVIII, in line with seta 1. There is an ellipsoidal copulatory papilla in the ventral mid-line between somites XIX and XX, and another between XX and XXI. The oviduct opens in somite XIV. There are two pairs of spermathecæ in somites VIII and IX, each having a small spherical appendage; their pores are on the anterior edge of the somite in line with seta 1. The ciliated rosettes of the sperm duct are in the somites preceding the seminal reservoirs, which lie in somites XI and XII. There is a large prostate in somite XVIII. There is no gizzard and no subneural blood-vessel.

Grube (30) has described, under the name Lumbricus littoralis, a worm from Villafranca, which is probably another species of Pontodrilus. This has three pairs of "copulatory papillæ," in line with setæ 2, in somites XIX, XX, and XXI.

In 1875 M. Perrier (32) described four species of Perichæta from the Philippines, and one from Cochin China.

P. bicincta has only two somites in the clitellum.

P. biserialis has the usual three somites in the clitellum; there is a ventral median and a lateral break on each side in the ring of setæ, and the ventralmost seta on each side is larger than the other setæ of the somite. There are several pairs of copulatory papillæ behind the male pores.

P. luzonica has a similar arrangement of setæ, but has the clitellum on four somites (XIV to XVII).

P. cœrulea also has the clitellum on three somites.

P. Juliana (from Saigon) has a continuous ring of setæ on each somite. It has four pairs of spermathecæ.

Grube described (32A) an Earthworm from Rodriguez, and named it Perichæta rodericensis.

Lankester, in 1879, described an Acanthodrilus from Kerguelen (33), A. kerguelenensis, in which the setæ are separated and form eight rows (but become paired in the genital somites); the male pores are in somites XVII and XIX; the spermathecæ are in somites VIII and IX; the penial setæ are notched. The nephridia of this genus are here mentioned for the first time.

Horst (31) has described a Perichæta from Java. Length 120 mm., consisting of 100 somites. The spermathecæ are four pairs in somites VI, VII, VIII, and IX, with their pores anteriorly. Each consists of a large ovoid sac with a neck, into which opens the duct of a much smaller, rather conical sac. The male pores are situated on papillæ, but no other papillæ are present. In other respects, it has the usual Perichætal structure; but in somite X (in three individuals) there was, on the left side, an unpaired blood-vessel passing from the dorsal to the ventral trunk.

In 1883 Horst described (34) nine Earthworms belonging to Schmarda's genus Perichæta, but he uses Templeton's name of Megascolex as having a priority. As they all appear to have a continuous ring of setæ in each somite, and no deficiency in the dorsal mesial line, mentioned by Templeton for his worm (20), it seems confusing to change the names: accordingly, in the list at the end of this section I have placed them in the genus Perichæta.

M. indicus, from Sumatra, may, according to the author, be the same as Schmarda's Perichæta cingulata.

M. sumatranus has the male pore, as usual in this genus, on somite XVIII, but here placed in a slight pit surrounded by a plicated wall.

M. Hasseltii, from Sumatra, has the setæ on each side of the ventral mid-line placed closer together than elsewhere in the ring of setæ.

M. Sieboldii, from Japan, and M. musicus, from Java, have six pairs of intestinal cæca in somite XXVI. Horst further describes M. capensis, from the Cape of Good Hope, and M. annulatus, from the Malayan Archipelago.

Hutton has described (35) two species of Baird's Megascolex (that is, Schmarda's Perichæta) with a continuous ring of setæ in each somite, from New Zealand.

M. sylvestris differs from all other species of the genus in having the male pores on somite XIX; there are sixty setæ round each somite, which are arranged in thirty couples.

M. lineatus has a continuous ring of setæ in each

somite, and in other respects agrees with other species of Perichæta.

In 1883 Mr. F. E. Beddard (36) described a worm to which he gave the name Pleurochæta Moseleyi; but he has since formed the opinion that it belongs to Templeton's genus Megascolex. It came from Kandy, in Ceylon. Its length is 28 inches; there are 260 somites, and about 140 setæ to each of them. The setæ are not in a continuous ring, but leave a space, ventrally and dorsally, along the median line; their length varies from ·035 to ·066 mm. The clitellum occupies somites XIII to XX. No nephridia were observed. The spermathecæ are in somites VIII and IX, with their pores in the anterior region. A pair of pores are present in each of the somites XIII, XVII, XVIII, and XIX. Those of somite XVIII belong to a pair of solid glands, which may perhaps be prostates, whilst the function of the other pores is quite unknown. The seminal reservoirs ("testes") are paired racemose glands in somite XII. Curious kidney-shaped glands open into the intestine in its anterior part.

In 1883 Beddard described (37) some new Earthworms from India.

Perichæta armata (Megascolex, Templeton), from Calcutta, has the clitellum in somites XIV, XV, XVI, XVII. There is a narrow break in the ring of setæ on the ventral surface of the somites. There are three pairs of spermathecæ in somites VII, VIII, IX. In somite XVIII, close to the male pore, on each side, is a sac containing a number of modified spiked setæ (as in Acanthodrilus).

Perionyx M'Intoshii, from Burmah, is 15 inches long. The male pores are not quite so close together as in P. excavatus, E. P.

In this paper, as also in the same Journal for 1884, he discusses Horst's proposal as to the limitation of the genera Perichæta and Megascolex; he also mentions that his genus Pleurochæta is identical with Templeton's Megascolex.

A new genus is formed, Typhæus, for an intrachitellian worm from Calcutta.

Ty. orientalis is ten inches long, and one third of an inch broad, and is cylindrical throughout. The setæ are in four couples, and are all on the ventral surface. The clitellum occupies somites XIV, XV, XVI, XVII. The male pores are in somite XVII, close together, on a flattened area. They are in a line with the ventral couples of setæ. Copulatory papillæ are present behind and in front of the male pores, on the intersegmental grooves (as in Pontodrilus).

In somite XVIII is a delicate sac containing modified penial setæ.

There is only one pair of spermathecæ, and they are in somite VIII. There are five pairs of lobed glands, lying above the dorsal blood-vessel, about the middle of the intestinal region. Nephridia were observed only in the somites anterior to the clitellum.

In 1884 Mr. Beddard mentioned in 'Nature' (38), and subsequently at the Zoological Society (39), that he had received a gigantic Earthworm from the Cape of Good Hope, for which he proposed the name Microchæta. I shall describe below two specimens apparently belonging to the same species.

In 1884 Horst described two species of Acanthodrilus from Liberia (43), A. Schlegelii, and A. Büttikoferi.

In 1885 Beddard described some anatomical points observed in some species of Acanthodrilus from New Zealand (40), where the dorsal blood trunk is double, and where there are eight nephridia to each somite (41), one corresponding to each seta; and another species where there are two alternating series of nephridia (42). Beddard also describes Ac. capensis (40) where the setæ are in four couples anteriorly, but separated posteriorly; he here, for the first time in this genus, finds the ovary in somite XII, and the oviduct with its pore in somite XIII.

TABLE OF THE CHARACTERS

	Genus.	Group.	Clitellum commences at Somite.	Number of Somites Clitellum extends through.	Position of Male Pore.	Copulatory Appendage.	Position and Number of Spermathecæ
A	Lumbricus, Linn.	Ante.	XXX	7	XV	...	IX and X
B	Hypogæon, Sav.	Ant. ?	XXVII	6 to 10	?	?	?
C	Megascolex, Temp.	Post.	?	?	?	?	?
D	Criodrilus, Hoffm.	A. ?	...	None	XIV	?	?
E	Helodrilus, Hoffm.	A. ?	...	None	XV	?	?
F	Echinodrilus, Vaill.	A. ?	...	None	XII	Pair of papillæ carrying male pores in line with ventral group of setæ	?
G	Perichæta, Schm.	Post.	XIV	3	XVIII	Sometimes papillæ, sometimes none.	V to IX
H	Pontoscolex, Schm.	Post.	XV	?	XIX	?	?
I	Tritogenia, Kinb.	A.	...	None	Between XVI and XVII	...	...
J	Mandane, Kinb.	Post.	XII	5	XVI and XVIII or XXI and XXIII	Four male pores	?
K	Geogenia, Kinb.	Intra.	IX	10	XVI	...	?
L	Alyattes, Kinb.	?	?	?	?	...	?
M	Eurydame, Kinb.	?	?	?	?	...	?
N	Hegesipyle, Kinb.	?	?	?	?	...	?
O	Rhodopis, Kinb.	Post.	XII	2	Between XIV and XV	...	?
P	Anteus, E. Perrier	Intra.	XV	15	? XI and XII	None	Pair in VII
Q	Titanus, E. Perrier	Intra.	XV	9	Between XVIII and XIX	None	None ?

OF THE GENERA OF EARTHWORMS.

Remarks on Spermathecæ.	Number of Setæ per Somite.	Arrangement of Setæ.	Position of Nephridiopore.	Length.	Habitat.	Date of Description.	Reference to Bibliography.	
From 2 to 10	8	4 couples	In line with 2nd setæ	4 to 6 inch.	Europe and N. America	...	...	A
...	9	Scattered	?	30 to 40 mm.	Buenos Ayres, and Sandwich Isles, &c.	1820	1	B
...	100	In a ring, with dorsal break	?	40 inches	Ceylon	1844	20	C
...	8	In 4 couples	?	8 to 12 inch.	Europe	1845	22	D
...	8	In 4 couples	?	2 to 5 inch.	Europe	1845	22	E
...	20	In 4 groups of 5	?	...	...	1851	23 and 24	F
From 2 to 8, either simple or with appendages	Very numerous	Equidistant	None	80 to 370 mm.	Various	1861	25	G
..	7	Alternating	?	70 mm.	Jamaica	1861	25	H
...	6	?	?	?	?	1866	19	I
...	8	Ventrally paired, dorsally scatterd	?	62 to 80 mm.	Patagonia and Montevideo	1866	19	J
...	8	In couples alternating anteriorly	In line with 3rd and 4th setæ	85 mm.	Natal	1866	19	K
...	8	In couples, but scattered posteriorly	?	?	Buenos Ayres	1866	19	L
...	8	(as above)	?	58 mm.	Panama	1866	19	M
...	8	Scattered, except anteriorly ventrally	...	28 mm.	Natal	1866	19	N
...	50 to 60	Equidistant	?	75 mm.	Java	1866	19	O
...	8	4 couples	In line with 4th setæ	1 met. 16 cm.	Cayenne	1872	14	P
...	8	4 couples scattered posteriorly	In line with 2nd setæ	1 met. 26 cm.	Brazil	1872	14	Q

TABLE OF THE CHARACTERS

	Genus.	Group.	Clitellum commences at Somite.	Number of Somites Clitellum extends through.	Position of Male Pore.	Copulatory Appendage.	Position and Number of Spermathecæ
R	Rhinodrilus, E. Perrier	Intra.	XIX	3	Between XIX and XX	A ventral groove between longitudinal bands	None ?
S	Eudrilus, E. Perrier .	Intra.	XIII	3 to 6	XVII	Strong curved chitinous penis in a sac	One pair in XIV
T	Acanthodrilus, E. Perrier	Post.	XIV	4	XVIII and XX	Four male pores, each with a group of penial setæ	VIII to X
U	Digaster, E. Perrier .	Post.	XIV	3	XVIII	...	VIII & IX
V	Perionyx, E. Perrier .	Post.	XIII	5	XVIII	A median pit	VIII & IX
W	Moniligaster, E. Perrier	A.	...	None	2 between VII and VIII, 2 between X and XI	...	None ?
X	Urochæta, E. Perrier .	Intra.	XIV	10	XX	None	VII, VIII, and IX
Y	Plutellus, E. Perrier .	Post.	XIV	4	XVIII	None	V, VI, VII, VIII, IX
Z	Pontodrilus, E. Perrier.	Post.	XIII	5	XVIII	Ellipsoidal papillæ in median line between XIX and XX and between XX and XXI	VIII and IX
A A	Allurus, Eisen . . (Lumbricus)	Ante.	XXII	4 to 6	XIII	...	...
B B	Typhæus, Beddard .	Intra.	XIV	4	XVII	Paired papillæ in front & behind the male pores.	VIII

OF THE GENERA OF EARTHWORMS.—Continued.

Remarks on Spermathecæ.	Number of Setæ per Somite.	Arrangement of Setæ.	Position of Nephridiopore	Length.	Habitat.	Date of Description.	Reference to Bibliography.	
...	8 ornamented	In 4 couples	In line with 3rd and 4th setæ	15 cm.	Venezuela	1872	14	R
It is united with ovary; opens in line with upper dorsal setæ	8	In 4 couples	In line with 3rd & 4th setæ	15 cm.	Antilles, Martinique, Rio Janeiro	1872	14	S
With small appendage	8	4 couples, or 8 separate	In line with 3rd & 4th setæ	10 to 35 cm.	New Caledonia and Madagascar	1872	14	T
Simple	8	4 couples	In line with 2nd setæ	...	Australia	1872	14	U
Pores near median ventral line	30	Equidistant	...	120 mm.	Cochin-China	1872	14	V
...	8	4 couples	In line with 3rd and 4th setæ	150 mm.	Ceylon	1872	14	W
...	8	4 couples anteriorly scattered and alternating posteriorly	In line with 3rd setæ	1 dcm.	Java, Brazil, &c.	1872 and 1874	14 and 28	X
...	8	Separate	Alternate with 2nd and 4th setæ	15 cm.	Pennsylvania	1873	27	Y
With small spherical appendage	8	1 and 2 are close together, 3 and 4 separate	In line with 2nd setæ	1 dcm.	France	1874	29	Z
...	8	4 couples	In line with 2nd setæ	35 to 50 mm.	Europe	1874	15	A A
Reniform, with small lobed sac on each side, opening into duct of spermathecæ	8	4 couples	...	10 inches	Calcutta	1883	37	B B

The following is a list of all Earthworms whose distribution is known, arranged according to Perrier's classification :

I. Anteclitelliani.

		Habitat.
Lumbricus	agricola, Hoffm.	Europe.
„	trapezoides, Dug.	Europe.
„	rubellus, Hoffm.	Europe.
„	chloroticus, Dug.	Europe.
„	olidus, Hoffm.	Europe.
„	complanatus, Dug.	Europe.
„	tetraedrus, Dug.	Europe.
„	puter, Eis.	Europe.
„	melibæus, Rosa	Europe.
„	purpurens, Eis.	Europe.
„	Josephinæ, Kin.	St. Helena.
„	Helenæ, Kin.	St. Helena.
„	Hortensiæ, Kin.	St. Helena.
„	infelix, Kin.	Port Natal.
„	capensis, Kin.	Cape of Good Hope.
„	novæ-hollandiæ, Kin.	Sydney.
„	Vineti, Kin.	Madeira.
„	victoris, E. P.	North Africa.
„	armatus, Kin.	Buenos Ayres.
„	tellus, Kin.	Buenos Ayres.
„	pampicola, Kin.	Montevideo.
„	Apii, Kin.	California.
„	tahitana, Kin.	Tahiti.
„	americanus, E. P.	New York.
„	uliginosus, Hutt.	New Zealand.
„	campestris, Hutt.	New Zealand.
„	levis, Hutt.	New Zealand.
„	annulatus, Hutt.	New Zealand.
? Alyattes, Kinb.		Buenos Ayres.
? Hypogæon, Sav.		Philadelphia.
? „	Atys, Kin.	Buenos Ayres.
? „	havaicus, Kin.	Oahu (Sandwich Isles).
? „	orthostichon, Schm.	New Zealand.
? „	heterostichon, Schm.	Quito and Cuença.

II. Intraclitelliani.

	Habitat.
Anteus gigas, E. P.	Cayenne (South America).
Titanus brasiliensis, E. P.	Brazil.
Rhinodrilus paradoxus, E. P.	Venezuela.
Eudrilus decipiens, E. P.	Antilles.
„ Lacazii, E. P.	Martinique.
„ peregrinus, E. P.	Rio Janeiro.
Urochæta hystrix, E. P.	Martinique, Gloria, Brazil, Java.
Typhæus orientalis, Bedd.	Calcutta.
? Geogenia, Kin.	Natal.

III. Post clitelliani.

Perichæta leucocycla, Sch.	Ceylon.
„ brachycycla, Sch.	Ceylon.
„ viridis, Sch.	Ceylon.
„ cingulata, Sch.	Ceylon.
„ posthuma, L. V.	Java.
„ ? sp., Horst.	Java.
„ cingulata, Sch.	Bourbon.
„ robusta, E. P.	Bourbon and Manilla (Philippines).
„ Houlleti, E. P.	Calcutta and Cochin China.
„ affinis, E. P.	Cochin China.
„ elongata, E. P.	Peru.
„ quadragenaria, E. P.	East Indies.
„ tahitensis, Gr.	Tahiti.
„ bicincta, E. P.	Philippines.
„ biserialis, E. P.	Philippines.
„ luzonica, E. P.	Philippines.
„ cœrulea, E. P.	Philippines.
„ juliana, E. P.	Cochin China.
„ rodericensis, Gr.	Rodsriquez.
„ sylvestris, Hutt.	New Zealand.
„ lineatus, Hutt.	New Zealand.
„ indicus, Horst.	Sumatra.
„ sumatranus, Horst.	Sumatra.
„ Hasseltii, Horst.	Sumatra.
„ Sieboldii, Horst.	Japan.
„ japonicus, Horst.	Japan.
„ musicus, Horst.	Java.
„ capensis, Horst.	Java.
„ annulatus, Horst.	Malay.
? Nitocris, Kin.	Rio Janeiro.

	Habitat.
? Amyntas, Kin.	Guam (East Indies.
? Pheretima, Kin. . . .	Tahiti and Ceylon.
? Rhodopis, Kin.	Java.
? Lampito, Kin.	Mauritius.
? Mandane, Kin.	Montevideo and Patagonia.
Megascolex cœruleus, Temp. .	Ceylon.
„ armata, Bedd. .	Calcutta.
Pleurochæta Moseleyi, Bedd. .	Ceylon.
Plutellus heteroporus, E. P. .	Pennsylvania.
Pontodrilus Marionis, E. P. .	Europe.
Acanthodrilus obtusus, E. P.	New Caledonia.
„ ungulatus, E.P.	New Caledonia.
„ verticillatus, E. P. . .	Madagascar.
„ kerguelenensis, Lankester .	Kerguelen.
„ capensis, Bedd.	Cape of Good Hope.
„ sp., Bedd. . .	New Zealand.
„ sp., Horst. .	West Africa.
„ sp., Horst. .	West Africa.
Digaster lumbricoides, E. P. .	New South Wales.
Perionyx excavatus, E. P. .	Cochin China.
„ M'Intoshii, Bedd. .	Burmah.

IV. Aclitelliani.

Moniligaster Deshayesii, E. P.	Ceylon.
? Tritogenia, Kin. . . .	
Criodrilus, Hoffm. . . .	Europe.
Helodrilus, Hoffm. . . .	Europe.

The genera marked ? are not distinctly enough characterised to be retained. I have not included in this tabular statement, and generally in this portion of the memoir, any details due to my own researches, which will be found in a subsequent section.

III. The Variations in the Structure of Earthworms Treated according to the Different Systems of Organs.

The Setæ.—Claparède (12) drew his characters for the Terricolæ as opposed to the Limicolæ, from Lumbricus, some of which characters are now known to hold only for that genus, others for only a few genera, and amongst them is the arrangement of the setæ in four groups of two setæ in each somite. This arrangement holds for many Earthworms, viz. Lumbricus, Anteus, Rhinodrilus, Eudrilus, Acanthodrilus, Digaster, and Moniligaster, including, therefore, forms from each of Perrier's groups (14).

In Urochæta, Titanus, and Acanthodrilus capensis, and in the doubtful Alyattes, and Eurydame of Kinberg, the same arrangement holds in the anterior part of the body, but varies posteriorly; the setæ become scattered in Titanus, Ac. capensis, Alyattes, and Eurydame, but remain in line, whilst in Urochæta they become scattered but alternate in consecutive somites.

In Ægesipyle, again one of Kinberg's doubtful genera, they are scattered (that is, the two setæ forming a couple become separated), except the anterior ventral couples.

In Acanthodrilus kerguelenensis the setæ are separate except in the genital somites.

In Pontodrilus throughout the body the ventral couple (setæ 1 and 2, counting from the mid ventral line) remain close together, but setæ 3 and 4 are separated.

Again, in Plutellus the eight setæ are nearly equidistant, but do not alternate, whereas in Pontoscolex there are only seven setæ in each somite, which alternate in consecutive somites throughout the body, and in Geogenia they alternate anteriorly only. In a form which I shall describe in a later paper, seven out of the eight alternate, whilst the ventralmost setæ (No. 1) remain in line throughout the body.

In Echinodrilus we have still four groups, but there are five setæ to each group.

In Hypogæon, Savigny described nine equidistant setæ, of which one was said to be in the mid dorsal line.

In Tritogenia, Kinberg, only six setæ are present in each somite. The number is greatly increased in Perichæta, where there may be as many as 100 to the somite, and in Perionyx there are thirty. These in each case are equidistant and form a complete ring round each somite.

In Megascolex, again, this arrangement is varied by an interruption in the ring in the mid dorsal line, whilst in Beddard's Pleurochæta, as well as this dorsal break, there is a similar break ventrally.

The setæ are not always simply pointed, as Claparède supposed, but in many cases are variously ornamented, as in Rhinodrilus, throughout the body; in the genital setæ of Urochæta and of Acanthodrilus; whilst in Urochæta all the setæ are bifid at their free extremity. Moreover they are modified in certain parts of the body, as Hering (5) has shown, for copulation: e. g. in Lumbricus, on clitellum, and on the somites XV and XXVI.

Pores.—Perrier has pointed out a sort of relationship between the nephridiopores and the couples of setæ; in some genera these pores are in front of and slightly dorsad of the ventral couples (setæ 1 and 2), as in Lumbricus, Titanus, Pontodrilus, whilst in other genera the pores have a similar relation to the lateral couples (3 and 4), Rhinodrilus, Eudrilus, Acanthodrilus, Anteus, Moniligaster [also in Microchæta].

Plutellus is exceptional at present in showing an alternation of the nephridiopores with the seta 2 and with seta 4 in consecutive somites, whilst the first few pairs are in line with the third seta. Again, they are, in Urochæta, related to the third seta throughout the body. They remain in this line even when the setæ alternate, though one would expect, if there were any relation between them and the setæ, that the nephridiopores would also alternate.

This difference in the position of the nephridiopores helps to confirm Prof. Lankester's theory as to the original presence of two pairs of nephridia in each somite, one series of which has disappeared in one set of worms, whilst the other series has gone in the second set, excepting in the genital somites, where they have been modified for the conveyance of the genital products to the exterior. This is well seen in such forms as Eudrilus, where the nephridiopore is in line with the lateral couple, and the male pore in line with ventral couple. In Anteus no sperm duct is known, but the nephridia are somewhat modified in the genital region, and may possibly serve as sperm ducts. It would be extremely important if it could be shown that such is really the case.

The pores of the spermathecæ are always anteriorly placed except in Eudrilus, where they are behind the seminal reservoirs [and in Microchæta, where they are still further back], and are rarely placed laterally as in Perichæta affinis, but more often in a line with the ventral setæ, as in Plutellus, Digaster, Pontodrilus, or as in Lumbricus, a little dorsad of these setæ. In Pontodrilus they are in a line with the first seta, in Plutellus they are in line with the second seta; again, in Urochæta they are in line with the lateral third seta. In Perionyx the pores are ventrad of the first seta.

The pores are either on the anterior or on the posterior edge of the somites, so close to the edge that they appear to be on the inter-segmental groove.

The Clitellum.—The clitellum has been taken by Perrier as the basis of his classification of the Terricolous Oligochæta, and it is only in a very few cases that it is absent.

Moniligaster has certainly no clitellum, although its genital organs are described as being fully developed.

Helodrilus and Criodrilus were described by Hoffmeister as having no clitellum, though sufficient details of the genital organs are not given by him, and the worms have not been studied recently.

The various genera belonging to the four groups, Anteclitel-

liani, Intraclitelliani, Postclitelliani, Aclitelliani, have already been given above.

The extent of the clitellum varies considerably in the same genus, even in the different specimens of the same species when fully developed; for instance, in Lumbricus agricola it occupies six or seven somites, XXX to XXXVI; in L. rubellus, somites XXVI to XXXII (seven somites); in L. trapezoides, somites XXVII to XXXV (nine somites); whilst in L. (Allurus) tetraedrus it occupies only six somites, from XXII to XXVII.

Amongst the Intrachitellian forms the same variation is to be noted: Anteus has as many as fifteen somites, XV to XXIX, making up the clitellum; Titanus has nine somites, XV to XXIII; Urochæta has ten somites, XIV to XXIII; Geogenia has ten somites; whilst Rhinodrilus has only three somites in this structure, XIX, XX, XXI. It commences as far forwards as somite IX in Geogenia.

In Eudrilus the three different species each have a different number of somites in the clitellum, E. decipiens having only three, XIII, XIV, XV; whilst E. Lacazii has six somites, XIII to XVIII, to form this organ. In Rhinodrilus the girdle is complete, whilst in the other forms it resembles Lumbricus in having a saddle shape.

Amongst the Postclitellian genera there are usually fewer somites occupied by the clitellum, which is a complete girdle, and nearly always commences with somite XIV (Perichæta, XIV, XV, XVI; Plutellus, XIV to XVII; Acanthodrilus, XIV to XVII, and others), or in XIII (Perionyx, XIII to XVII, and Pontodrilus, XIII to XVII); in XII, in Kinberg's genera Rhodopis and Mandane, or in XV, in Pontoscolex. (In Kinberg's genera, Nitocris, Amyntas, Lampito, Pheretima, the clitellum appears to commence at somite XIV, and this character with the numerous setæ to each somite would justify us in considering them as forms of Perichæta.)

Rhodopis, if Kinberg's description can be considered of any value, possesses the shortest clitellum, occupying only two somites, as is also the case in Perichæta bicincta.

The position of the clitellum and the position of the gizzard

seem to have some sort of relation to one another, for in the Anteclitellian worms, where the clitellum commences somewhere about XXV or further back, the gizzard is behind the genital organs. But in the Postclitelliani and Intraclitelliani the gizzard passes forwards to somites VI or VII, and is usually in the same somites with or in front of the seminal reservoirs.

In the forms which have not a complete clitellum there is very frequently a glandular ridge on each side of the ventral surface, along or near the ventral edge of the clitellum. This is seen in Lumbricus, where Eisen (15) speaks of it as "tubercula pubertatis." Perrier figures the same sort of ridge in Rhinodrilus, but whether it occurs in other forms I do not know. It very probably does so as it would appear to have some function in copulation.

The true limits of the clitellum anteriorly and posteriorly are not always evident, as the thickening of the epidermis is gradual, and frequently is apparent on the dorsal surface before it is so ventrally.

Dorsal Pore.—In many Earthworms the cœlom is put into communication with the exterior by means of a series of "dorsal pores" placed on the intersegmental grooves. In Lumbricus these pores occur in every somite after about somite VIII. In Digaster and Perionyx they commence just behind somite IV. In Plutellus behind somite VI. In Pleurochæta and Typhœus the pores are present only behind the clitellum. They are present in Acanthodrilus, and in many Perichætæ.

The Alimentary Tract.—The main regions into which the digestive canal is divided are constant in all the Earthworms. There is a pharynx, œsophagus, gizzard (except in Pontodrilus), and an intestine.

The pharynx is a strongly muscular organ and of glandular appearance, though in Lumbricus and others I can find no glands in the wall. Perrier has found glands in the pharynx of Pontodrilus. The anterior part of this organ has quite thin walls, and is in some at least capable of slight protrusion. This thin-walled region is the "buccal" region,

and extends back as far as the circumpharyngeal nerve commissure.

In Perichæta the pharynx is provided with three pairs of glands, which open into it, in Moniligaster also there are small glands in this region. In other cases, e.g. Urochæta, the first pair of nephridia are much modified, their tube enlarged and glandular, and the coils are flattened against the walls of the pharynx so as to become more or less buried in its muscles, but they have no opening into the pharynx.

The pharynx usually occupies four or five somites, though, owing to the somewhat infundibulate septa, it appears to occupy more somites. Lumbricus is often described as having the pharynx extending through seven somites, whereas really it occupies only five. In Pontodrilus it occupies only three somites.

The œsophagus varies much in length, occupying twelve somites in Lumbricus (including the exceptional "proventriculus" of this form) whilst in Eudrilus it only extends through two somites. In Digaster, owing to the presence of a second gizzard, there is a second œsophagus between them.

In the greater number of genera there are no glands in this portion of the alimentary tract; e.g. in Eudrilus, Rhinodrilus, Acanthrodilus, Digaster, Perionyx, and others. But in Lumbricus there are three pairs of enlargements known as "œsophageal" or calciferous glands, two being in somite XII, the third in somite XIII. The two anterior contain a milky liquid; the last contains solid carbonate of lime.

Anteus also possesses "œsophageal glands." But it is in Perichæta that the glandular appendices of the alimentary tract are most fully developed. Besides the three pairs of pharyngeal glands there are three different sorts of glands to the œsophagus. In each of the somites, VI, VII, VIII, is a pair of tufts of glandular tubules. In somite VI there is a pair of large ovoid, solid-looking glands, each of which opens by a distinct pore into the œsophagus, whilst in somite VII there is a pair of grape-like glands.

The great development of these glands in Perichæta seems related to the extremely small size (? absence in some) of the nephridia. Perrier has suggested that they act in some way as excretory glands, the excretion, however, being used as a digestive fluid, instead of being passed directly to the exterior, just as the liver of Vertebrates is in a way an excretory gland.

The gizzard is present in all but Pontodrilus. The walls are very muscular, and the lining epithelium secretes a chitinous lining, which forms the crushing apparatus.

The gizzard is situated very far back in Lumbricus, where it occupies somites XVII and XVIII. Here it is quite behind all the genital organs even behind the male pore. In Perionyx the gizzard is in somite XII, in the same somite as the posterior seminal reservoir.

In all the other forms it is in front of the seminal reservoirs, being usually in somite VI (Anteus, Titanus, and Plutellus), or in somite VII (Urochæta, Eudrilus, and Rhinodrilus). In some cases it occupies only one somite, in others it occupies more than one. In Anteus the anterior septa are very thick and infundibuliform, covering the gizzard; but frequently the septum immediately in front of this organ is thinner than the neighbouring ones. In Perichæta the somite IX contains the gizzard. Digaster is so named from its possessing two gizzards, the anterior being in somite V, the posterior in VII, the portion of the alimentary tract between them being the second œsophagus. A further complication I have found in a worm from St. Thomas's, where there are three separate gizzards. Moniligaster again has a gizzard in somite VI of the usual form, whilst an elongated gizzard occupies somites XIII to XXII inclusive, being constricted into four portions. Here, therefore, the first gizzard is in front of the true genital organs, the second being behind them.

The Intestine following the gizzard is frequently separated into two regions, the portion directly behind the gizzard being the "tubular," and following this a more or less "sacculated" region where the typhlosole is developed.

In Lumbricus this tubular portion occupies only two

somites, XIX and XX; whilst behind this comes the sacculated, typhlosolar region, with thin walls covered externally by the large, yellow cœlomic epithelium; in this form the typhlosole is well developed, and, as usual, presents a blood-vessel in frequent connection with the dorsal trunk.

In other forms the tubular portion has a greater extent; for instance, in Anteus it extends from about somite VIII to XVIII, in Titanus from somite VI to XV. In Acanthodrilus, judging by Perrier's figures, one species has none, in another this region extends to somite XX, and in Urochæta as far as somite XV. In all cases where a structure is said to extend to a certain somite, it is understood to exist in that somite as well as in the preceding somites.

In these forms œsophageal glands are absent, but their analogue seems to be frequently present in the form of one or more pairs of glands, which contain carbonate of lime, situated on the tubular region of the intestine.

In Urochæta there are three pairs of such glands, elongated and ovoid, in somites VIII, IX, X; in Plutellus three pairs of glands of the same nature occur in somites X, XI, XII, but are reniform.

In Titanus is a single pair of white, nearly spherical glands, in somite XIII, which Perrier mistook for a part of the vascular system. He describes it as a "ventricle." I have had the good fortune of dissecting a Titanus, and have seen distinctly the large openings of these glands into the intestine.

One of the characteristics of the genus Perichæta appears to be the presence of a pair of elongated cæca, springing from the ventral surface of the sacculated intestine in somite XXVI or XXVII. In P. Sieboldii, Horst, there are six pairs of cæca in somite XXVI. Although Perionyx resembles Perichæta in many points these cæca are absent in it.

The typhlosole is usually a sub-cylindrical longitudinal valve produced by an involution along the dorsal wall of the sacculated intestine; but in Titanus this organ is flattened from side to side, whilst in Pontodrilus the vessel alone exists. Pleurochæta has no typhlosole and possesses a

series of reniform glands in the posterior part of the tract. Typhæus (37) possesses lobed glands on the middle region of of the intestine.

The sacculated portion of the intestine appears to be similar in all these worms, and is continued to near the end of the body when the typhlosole disappears, and the region is called rectum.

The structure of the glands, whether they occur on the œsophagus or on the tubular intestine, is very similar in some of those that have been investigated.

Claparède (11) has described and figured a section of the œsophageal gland of Lumbricus, where it consists of numerous alternate blood-vessels and glandular tubules placed radially.

Perrier describes the "glandes de Morren" of Urochæta as having the same structure, but his figure does not quite agree with the text, as he figures no blood-vessels. In Titanus the gland has the same structure [and I shall show that in a worm (Microchæta), to be described further on, the structure of the intestinal gland resembles that of the œsophageal gland of Lumbricus' very closely]. But the various œsophageal glands in Perichæta each have a distinct structure, and none seem, from Perrier's figures, to resemble the abovementioned glands.

In all the above glands (except Perichæta) carbonate of lime has been found; usually solid, but sometimes in the form of a milky fluid. Hence we have analogous glands in various regions of the alimentary tract, and in different somites. So also the gizzard occurs in different somites. Perrier has suggested that these may not only be analogous but even homologous organs: the worm being made up of somites, each of which somites was originally exactly alike, in one somite of one worm a part of the alimentary tract becomes a gizzard, whilst in a second worm the modification occurs in a different somite. But each of these gizzards is a modification of an originally homologous organ, therefore the gizzards are homologous. In the same way the glands are modifications and swellings at different parts of the alimentary tracts, which were

originally homologous; therefore these glands, whether œsophageal or intestinal, are homologous. This view cannot, however, be held if we apply the only true test of homology, that of common origin from a common ancestor. It is quite clear that a gland which is in somite XXIV cannot be the same thing as a gland which existed in somite XIII of an ancestor, or vice versâ. If we are to suppose that similar parts have been similarly modified for similar wants in different somites, of two genera of Earthworms compared, then the case is one, not of homology, but of "homoplasy." (See Lankester, 44.)

The Nervous System.—This seems very similar in all the worms studied, consisting of a pair of supra-pharyngeal ganglia, and a series of ventral ganglia, united by cords; besides these, in at least some forms (Urochæta, Perichæta, Lumbricus, and others), there is a visceral system of cords and ganglia on the pharynx, and probably continued farther backwards: these originate partly from the supra-pharyngeal ganglia and partly from the circum-pharyngeal commissures.

The presence of the "three great fibres" in the ventral cord appears pretty constant; but the sub-neural vessel is not so universal as Claparède supposed; for in Pontodrilus and Perichæta Houlleti, Perrier has shown that this vessel is absent [as I shall show later on to be the case in at least one other worm Microchæta], and as Beddard has shown to be the case in Pleurochæta. Perrier considers that the supra pharyngeal ganglia are always in somite III, but he is wrong, for in Titanus these ganglia lie in somite II, although dragged back by the muscles of the pharynx; the first ventral ganglion lies in somite III, and it is usual to find that ganglion in the somite following that in which the supra-pharyngeal ganglia lie.

[In Microchæta and other Earthworms which I shall describe below, the supra-pharyngeal ganglia lie in somite I distinctly.] Undoubtedly, most frequently it is the somite III that in the adult is occupied by the supra-pharyngeal ganglia, although their seat in embryological origin is the prostomium.

The Vascular System.—In its simplest form, as in Peri-

chæta, the closed vascular system consists of a dorsal and a ventral longitudinal trunk, together with a typhlosolar trunk in the intestinal region. In the anterior region of the body, paired commissural vessels or "lateral hearts" connect the dorsal with the ventral trunk. In Pontodrilus, a pair of longitudinal lateral trunks ("intestino-tegumentary" vessels of Perrier) are added to these three: these lateral trunks rise from a capillary network on the alimentary tract and pass forward to a similar network on the wall of the pharynx; these lateral trunks are nowhere in direct communication with either the dorsal or the ventral trunk. The lateral longitudinal trunks occur in Lumbricus, where they rise as a pair of branches from the dorsal trunk in somite x. In Urochæta [in Microchæta], and possibly in Pleurochæta, they have the same arrangement as in Pontodrilus. These trunks have not been described in other genera. There is a subneural trunk in all forms, except in Perichæta, Pleurochæta, Pontodrilus [and Microchæta].

From these longitudinal trunks, of which the dorsal and ventral are chiefly contractile, paired vessels pass to the septa, nephridia, body wall, and alimentary tract; in these organs they break up into networks of capillaries, whence the blood is again collected by vessels which returns it to the main trunks. In Perichæta and Perionyx Beddard has shown that there are capillaries in the epidermis itself similar to those known in the leech. Howes has figured in the 'Biological Atlas' a small "infra-intestinal" vessel, closely attached to the ventral wall of the intestine in Lumbricus; this I have not myself seen, nor is it mentioned by M. Jaquet (49) in his description and figures of injected specimens of this genus. But Mr. Beddard has informed me that he has seen it in Acanthodrilus. Besides the "lateral hearts" in the anterior somites, there may be also commissural vessels in the intestinal region. These in Lumbricus pass from the dorsal to the subneural trunk. I have found that the organ in Titanus, which Perrier regarded as part of a lateral heart and called "ventricle," is really an intestinal gland.

The dorsal trunk is always muscular. It may be simply tubular in Lumbricus, Moniligaster, Perichæta, Titanus, and others, or it may be ampullate and valvular, as in Urochæta throughout the body; or tubular posteriorly and ampullate anteriorly as in Anteus; in both these last forms the dorsal trunk is bent to one side in a loop, just before the region where it gives off the lateral hearts.

In Pleurochæta the dorsal trunk divides in some of the anterior somites to form a double vessel, the halves of which unite again; there appears also to be a second dorsal vessel below the large contractile trunk. Beddard (40) has described a somewhat similar arrangement in a species of Acanthodrilus and in Microchæta.

Longitudinal lateral trunks appear at each side of the alimentary tract anteriorly in some worms. In Lumbricus these are formed by a branch from the dorsal trunk on each side in somite x. In Urochæta and Pontodrilus [and in Microchæta] (and doubtfully in Pleurochæta) they spring from a network in a part of the alimentary tract and run through eight or nine somites to the pharynx.

The lateral hearts usually occupy the somites in which the seminal reservoirs are, and those just anterior to them.

In Urochæta and Anteus there are three pairs, in somites VIII, IX, X; in Plutellus, in somites X, XI, XII; in Rhinodrilus, in somites XVII, XVIII, XIX; whilst in Lumbricus, Digaster, and Titanus, they exist in somites VIII to XII; in T. forguesii, in somites XI, XII, the hearts communicate with dorsal and typhlosolar trunks. In Pleurochæta and Perichæta the lateral hearts are in somites X to XIII, and in Pontodrilus they are in somites V to XI. These lateral hearts pass from the dorsal to the ventral trunk, but other vessels exist in some forms, passing from the typhlosolar to the ventral trunk. These "intestinal hearts" are present in somites XX, XXI, XXII in Rhinodrilus just behind the lateral hearts, and in Urochæta in somites XIII and XIV; in this latter worm they are very much larger than the lateral hearts, whilst in Pontodrilus the two intestinal hearts

in somites XII and XIII communicate both with the dorsal and with the typhlosolar trunks on the one hand and with the ventral trunk on the other. Thus in the Anteclitelliani the hearts are anterior to, whilst in the other groups they are either posterior to the gizzard or in its neighbourhood.

The subneural trunk is absent in Pleurochæta, Perichæta, and Pontodrilus [and in Microchæta].

The Course of the Blood.—The blood passes forwards along the dorsal (and typhlosolar) trunks and backwards along the ventral (and neural) trunks. The intestinal vessels rising from the dorsal trunk carry blood to the wall of the intestine, where there is a very close network of vessels, usually made up of longitudinal and circular branches; from this capillary network the blood is carried into the ventral trunk. From the ventral trunk a pair of vessels in each somite carries blood to the septa, nephridia, and body wall, where it is distributed in delicate loops and collected again by vessels which enter the dorsal trunk. This seems to be the course of the blood, as determined in Microchæta, where valves are placed in the dorsal trunk, at the exits and entrances of the vessels.

Anteriorly the blood is distributed over the wall of the pharynx and collected from the network into the ventral trunk. In the lateral hearts the blood passes downwards from the dorsal to the ventral trunk.

In the longitudinal lateral trunks the course of the blood seems to vary. In Lumbricus, it is evident that it passes forwards to be distributed over the pharynx, whence it is collected by the branches going to the ventral trunk. In Urochæta, Perrier considered this forward direction preferable; while in Pontodrilus he thinks it probable that the blood has not the same direction as in the dorsal trunk.

The Blood, by which I refer to the red liquid in the closed system of vessels, is a liquid, coloured red by hæmoglobin, in which float oblong colourless corpuscles, as has been shown by Lankester in 1878 to be the case in Lumbricus (45), and by Bourne and Blomfield in 1881 for the Polychæta (46).

These corpuscles can readily be seen by killing a piece of tissue, such as a septum or a nephridium, with $\frac{1}{10}$ per cent. osmic, and then staining in picrocarmine.

The Nephridia.—These have been figured by Gegenbauer (10) and described histologically by Claparède (11) for Lumbricus. Each nephridium or "segmental organ" of Dr. Williams (47) is a more or less coiled tubule with an internal funnel-shaped opening at one end, and an external pore at the other. The tube itself is divisible into three regions, the innermost leading from the funnel is cilated internally; this leads to a glandular region, and this to a short, muscular, slightly enlarged "vesicular" region. The lumen of the first two regions is intracellular, whilst that of the vesicle is intercellular and surrounded by muscle-fibres. The histology of the nephridia has not been minutely studied in any form, except in Lumbricus.

Nephridia are at present known in nearly all the forms whose internal anatomy has been described.

In Digaster Perrier appears not to have detected the organ or its pore. Beddard did not find them in Pleurochæta.

In most of the Perichætas they are so small as to have led to the impression that they are absent, but in P. robusta and P. affinis delicate tubules are attached to the septa, but Perrier gives no details. [In a Perichæta from the Philippines I have found numerous small nephridia in each somite by means of sections. Beddard informs me that he has made a similar observation.]

In the worms which possess nephridia the internal funnel is usually situated in the somite anterior to that in which the tubule lies, but in Plutellus the whole organ lies in one somite, and it has a large vesicular portion.

In Typhæus the nephridia have only been observed in the anterior somites.

In Titanus the nephridia do not commence till somite XIV.

In Anteus those of the clitellar region are shorter, wider, and less coiled than the others, and are supposed by Perrier to function as sperm ducts.

In Urochæta the first pair of nephridia are much modified and consist of a rosette of tubules opening into a large vesicle, and closely applied to the pharynx.

[The greatest development seems to take place in Microchæta, where all the nephridia have the structure of the first nephridium of Urochæta, but have an immense vesicular region. Other Earthworms, that I shall describe later, also have large elongated vesicles, and comparatively short glandular region.]

In Pontodrilus the anterior nephridia seem to be simple tubes, but in those posterior to the genital organs an immense compact glandular region is added, whilst the free portion of tubule is short.

In Moniligaster the nephridia are rather smaller in the genital somites than elsewhere.

As to the pores of the nephridia, the position of which relative to the setæ is an important generic character, we have more information.

Kinberg, as a rule, makes no mention of them, but in describing Geogenia he says the "lateral pores" are in a line with the dorsal setæ.

I have already mentioned the position of these pores in the various genera in describing the external features. As a rule, they are placed in the anterior region of the somites, just in front and slightly dorsad of either seta 4 or seta 2 (the upper seta of the lateral or of the ventral couple). But in Urochæta they are in this relation to seta 3 (the lower seta of the lateral couple), whilst in Plutellus they alternate between setæ 4 and 2, and the first five are in line with seta 3.

In a species of Acanthodrilus from New Zealand, Beddard has described (42) the position of the nephridia as alternating from somite to somite with the two couples of setæ; coinciding in one somite with the lateral, in the next with the ventral, and again, in the succeeding somite with the lateral couple of setæ. Moreover, these two sets of nephridia are different from one another; the ventral nephridium has a large diverticulum, whilst the dorsal one has a very small diverticulum. This fact,

as he points out, is additional evidence in favour of Lankester's theory of there having been originally two pairs of nephridia to each somite.

The nephridia in Lumbricus and others that have been studied are very well supplied with blood-vessels, which from time to time have dilatations on them, in which are frequently numerous blood-corpuscles; these have been figured by Lankester (48) and Claparède (11). These dilatations, however, are not confined to the nephridia, they occur on the vessels of the septa [and are particularly abundant on the grape-like pharyngeal glands of a new genus Trigaster].

Genital System.—Throughout this description, as also in future papers, I use the word "seminal reservoir" for the organs that Perrier and others call "testes" or "testicules." The male generative organs consist of one or more pairs of seminal reservoirs, the sperm ducts and their "ciliated rosettes" (or funnels). The true testes are probably always hidden in a mature worm within the substance of the seminal reservoirs, though this is only known to be the case in Lumbricus [an Microchæta]. Besides these more important organs various accessory copulatory appendages are known.

The Testes.—In Lumbricus the testes are four small plate-like masses of large cells attached to the anterior septa of somites x and xi, close to the nerve cord in a similar position to that of the ovary in somite xiii. They can only be observed in a worm in which the clitellum is undeveloped.

[In Microchæta the two pairs of testes exist in two pairs of horn-like, hollow prolongations of the anterior median part of the seminal reservoirs; these prolongations have thick walls, and I think they are very probably permanent.]

The Seminal Reservoirs.—As a rule these are sac-like, more or less compact, whitish bodies.

In Titanus there is only a single pair of seminal reservoirs of the same general appearance as the two pairs in Lumbricus, but they are prolonged through twelve or fifteen somites on each side of the alimentary tract. [I shall describe another

worm, Urobenus, *n. g.*, in which the single pair of seminal reservoirs extends through thirty or more somites.]

In Urochæta, too, there is but a single elongated pair, occupying somites XIII, XIV, XV, as is also the case in Typhœus. In Rhinodrilus there is but one pair, spherical in shape. But the most usual number seems to be two pairs, and though Perrier describes three pairs in Eudrilus decipiens (for instance), I fancy that the third pair are really only prolongations of the other reservoirs such as we have in Lumbricus.

Two pairs occur in somites IX and X in Anteus and Eudrilus; in somites X, XI in Lumbricus;[1] in somites XI, XII in Pontodrilus, Perionyx, in Perichæta and Acanthodrilus ungulatus. In all these forms the reservoirs are very like those of Lumbricus, but in Moniligaster there are two pairs of seminal reservoirs, of which those in somite VIII are very small and globular, whilst those in somite X are much larger; the whole generative system in this worm is very complicated and curious. In Digaster are two pairs of grape-like organs in somites X, XI, which Perrier considers as "testicules," but whether they are testes or seminal reservoirs is not apparent.

In Plutellus in somite XII, and in Acanthodrilus obtusus in somite XIII, there is a pair of grape-like organs, which contain bundles of developing spermatozoa. In Pleurochæta there are several enigmatical structures in the genital region, but whether the grape-like organs in somite XII are the same thing as the smooth-looking seminal reservoirs of better-known worms seems to me uncertain, as Beddard says nothing of their structure.

[1] In Lumbricus the rudiments of the seminal reservoirs appear on each side as an anterior growth of the septum between the somites IX and X, as a similar pair of anterior outgrowths on the septum between X and XI, and a further similar pair of posterior outgrowths on the septum between XI and XII. There are thus three pairs of saccular rudiments, on each side: of which the two anterior pairs unite to form a single organ in somite X, whilst a similar union takes place between those of the right and left sides in somite XI.

The sperm duct in Earthworms opens internally by a funnel, or "ciliated rosette." Usually the sperm duct, which is single at the external pore, becomes double anteriorly, and ends in a ciliated rosette beneath each seminal reservoir situated in the same somite; this ciliated rosette is usually enclosed in the reservoir, and though Perrier describes it as sometimes free, e.g. in Perichæta Houlleti, yet it is probably so only in the immature worm.

Only in one case, Pontodrilus Marionis, are the ciliated rosettes in front of the seminal reservoirs, being here in somites x, xi, whilst the reservoirs are in somites xi, xii. Another variation comes about by the presence of four separate sperm ducts, each with its external pore, as in Acanthodrilus and Moniligaster. In Anteus no sperm ducts are known, and the nephridia of the clitellar region are supposed by Perrier to function as sperm ducts.

Accessory Organs.—It is only in a few genera that the sperm duct is without a gland or enlarged portion near the pore, e. g. Lumbricus, amongst the Anteclitelliani, Urochæta [as well as Microchæta, and other forms that I shall describe later on], amongst the Intraclitelliani.

In Titanus the sperm duct opens into an enlarged, flat, muscular sac, which does not seem capable of protrusion. There is no gland, or "prostate" as it is usually called.

In most forms, whose setæ have been examined, it is found that those on the somite at which the male pore opens are more or less modified. Thus, in Lumbricus they are slightly modified; in Rhinodrilus they are elongated and ornamented, as they are also in Urochæta.

In Acanthodrilus this modification is carried further. At each of the four male pores is a bundle of setæ, usually recurved, enclosed in a sac opening close to the male pore, and the distal portion of the doubtful prostate is muscular, and probably protrusible. The most complete "penis" is found in Eudrilus, where a strong, recurved chitinous hook is enclosed in a spherical sac on each side of somite xvii, where the male pore opens. Opening into this sac, besides the sperm duct is an

elongated "prostate" (which Perrier calls "seminal vesicle"). Beddard has shown similar sacs of modified setæ in Typhæus and Perichæta (Megascolex) armata (27).

In Perichæta and Perionyx the prostate is present in the form of a more or less lobed or incised gland, opening by a duct into the sperm duct, whilst Moniligaster, Pontodrilus, and Plutellus have a tongue-shaped prostate similarly situated, and the common duct is muscular, curved, and probably protrusible.

In Pleurochæta each of two pores in somite XVIII leads into a solid white gland, which from its position appears to be a prostate.

The male pore is situated, usually, about the somite XVII or XVIII, but in Lumbricus it is in somite XV. As has been mentioned above, the relation of this pore to the clitellum is used to group the Earthworms, and the absence or presence of papillæ at or near these pores may be used to subdivide some of the genera. Lumbricus has its pores on wide but not prominent papillæ.

In most Perichætæ there are similar copulatory papillæ. Thus, in P. robusta a pair of papillæ are placed on somite XVIII between the male pores. P. affinis has the male pores on papillæ, as well as a pair of papillæ in somite XVII and a pair in somite XIX. In P. aspergillum the papillæ carry, besides the male pores, numerous smaller pores corresponding to small, internal, globular sacs. Similarly, round the pores of the spermathecæ (in this species) are other pores opening into sacs internally.

In P. Houlleti, quadragenaria, and elongata no copulatory papillæ are described.

In Megascolex sylvestris (Hutton) the male pore is in somite XIX.

In Perionyx, Pontodrilus, and Pleurochæta the pores are situated in pits in somite XVIII, which in the case of Perionyx are close together near the middle line, and not, as usual, latero-ventrally. In Typhæus the male pore is in somite XVII, and copulatory papillæ are present. In Rhino-

drilus the pores of the sperm ducts are in pits, the edges of which are produced backwards as a ridge, on each side of somite XX.

There are four male pores in Moniligaster, one pair between somites VII and VIII, and one pair between somites X and XI; similarly in Acanthodrilus one pair is on somite XVIII, and one pair on somite XX. From the sides of each of the latter the bunches of penial setæ project.

The Female Organs.—These consist of the ovaries, oviducts, and spermathecæ.

The ovaries are usually of small size and are frequently overlooked; for instance, in Titanus brasiliensis Perrier could find none, but he mentions a pair in somite XVIII in T. forguesii (29, p. 235).

The ovaries are a single pair; they are always placed behind the seminal reservoirs, but in Plutellus a pair of grape-like organs, supposed to be the ovaries, are situated in somite X, in front of the male organs.

In shape they are pyriform in Lumbricus (XIII), grape-like in Plutellus (X), Pontodrilus (XIII), Perionyx (XIII), [Microchæta (XIII)], tongue-shaped in Moniligaster (XII to XV), though it is doubtful whether these exceptionally large organs in the last genus are ovaries.

In Perichæta they are flat, pedunculated structures situated in somite XIII.

In Acanthodrilus it is doubtful whether the lobed organ described by Perrier in somite IX is an ovary or not; but Beddard has described (40) the ovary of Ac. capensis in somite XIII.

In Eudrilus the ovary, according to Perrier, is very exceptional; it is a globular sac, which is sessile on a supposed "spermatheca" in somite XII or XIV. The ovary has not been found in Anteus, Rhinodrilus, Digaster, nor in Urochæta, and is very doubtful in Pleurochæta and some of the above genera.

The oviduct is known in still fewer forms, though its pore is sometimes noticeable. In Lumbricus it is a short, rather

wide duct, opening interiorly into somite XIII and exteriorly in somite XIV; it is similar and similarly placed in Pontodrilus and Perichæta, and Acanthodrilus capensis; in Plutellus it is in somite X. In Eudrilus the neck of the so-called "spermatheca" acts as oviduct, according to Perrier. In Moniligaster the oviduct is exceptional in being in continuity with the ovary and opening in somite XII. In other forms it is unknown, though frequently, as in Pleurochæta [and in Microchæta], funnels are known which may have this function. The pores of the oviducts are in line with the ventral setæ, except in Perichæta and Perionyx, where they are median.

It is interesting to note that the oviduct in Plutellus opens interiorly and exteriorly in the same somite, as the nephridia of the same animal do, whilst in other forms both organs pass through a septum.

The Spermathecæ.—These organs, being large and frequently complicated, are well known in nearly all the genera, and they may be used to subdivide the genera. They are in all cases but Eudrilus [and Microchæta, where they are twenty or more horse-shoe shaped organs, in 4 pairs of groups opening in a row] in front of the seminal reservoirs. In the former they consist of a pair of pyriform sacs seated on a long coiled duct, in somites XII or XIV. The spermathecæ are usually globular or pyriform, and open laterally, dorsad of the ventral setæ, though they sometimes, as in Pontodrilus, are in a line with the ventral (1 and 2) setæ, and sometimes quite lateral as in Perichæta affinis. Even in the same genus they vary a great deal in number and in shape; Lumbricus subrubicunda has only a single pair in somite X, opening at the anterior edge of the somite, whilst L. complanatus has seven pairs, in somites VI to XII, opening at the posterior edge; L. agricola has two pairs in somites IX, X, opening posteriorly, and L. chlorotica three pairs, in somites IX, X, XI, opening anteriorly; there are other variations in this genus, but the spermathecæ themselves are all simple, globular, sessile sacs. In Typhæus there is only a single pair of spermathecæ in somite VIII.

In Anteus, Titanus, Rhinodrilus, no spermathecæ are known, nor are they certainly known in Moniligaster, but the genital system is so complicated and peculiar in this form that it demands further study.

In Acanthodrilus, Pontodrilus, Digaster, Pleurochæta, there are two pairs of spermathecæ in somites VIII and IX, opening anteriorly; in the last form are they bilobed, each having a smaller sac-like protrusion from its side as in Perichæta. In Perionyx, the spermathecæ are two pairs of simple sacs, in somites VII and VIII, open to the exterior close to the middle line.

In Urochæta the spermathecæ are also simple pyriform sacs, six in number, lying in somites VIII, IX, X, and opening in a line with the third setæ.

In Plutellus the spermathecal pores are in a line with the third setæ; the spermathecæ are in somites V, VI, VII, VIII, and IX, open anteriorly, and each consists of a sac and a coiled accessory portion.

It is in the genus Perichæta that these organs become most complicated. In P. elongata there is only a single pair, opening between somites IV and V; each consists of a simple sac. In P. affinis, P. cingulata, and P. posthuma, each spermatheca has a small secondary globular sac at its side, and there are four pairs placed in somites VI to IX. In P. quadragenaria and in Horst's species from Java, there is a narrow coiled tube opening into the neck of the spermathecal sac; in the former there is only one pair of spermathecæ, and they are placed in somite VIII.

In P. Houlleti the spermatheca consists of three parts, a large sac, a coiled tube, and a smaller sac.

In all these cases these parts have a common external pore, but in P. robusta there are these three parts, of which the small sac-like portion opens separately by means of a pore, just behind the true spermathecal opening. Again in P. aspergillum the spermatheca has numerous very small sacs around it, each having a separate pore to the exterior.

It seems to me that the numerous species of Perichæta may

be conveniently grouped, firstly by the presence or absence of "copulatory papillæ" at or near the genital pore, and then again grouped according to the number of their spermathecæ. The number of setæ per somite is perhaps rather variable in individuals of the same species—at any rate, they differ in the anterior and posterior somites.

Evidence is continually accumulating for Lankester's theory of the presence originally of two pairs of nephridia in each somite, and the modification of those of one series, in the genital somites, to serve as genital ducts.

In Lumbricus, Titanus, Pontodrilus, the ventral series of nephridia persist, their apertures being in relation to the ventral setæ. In these forms, then, the dorsal series of nephridia has disappeared, except in the genital region; that is, the oviducts are modified dorsal nephridia, the sperm ducts, and perhaps also the spermathecæ, are also dorsal nephridia which have shifted their position.

In Rhinodrilus, Eudrilus, Anteus, Urochæta, Moniligaster [and Microchæta], the ventral series, except in the genital somites, have disappeared, the dorsal series remaining as nephridia. In Anteus it seems probable that a very slight modification of some of the dorsal nephridia in the clitellar regions enables them to perform the function of a sperm duct, as no distinct sperm duct is known.

In one species of Acanthodrilus the dorsal and ventral series of nephridia are alternately suppressed (42), as Beddard has shown, whilst the same arrangement has been shown by Perrier to be present in Plutellus.

So far as the oviduct is concerned, the homology is fairly obvious, since it opens internally in one somite, passes through the posterior septum and opens externally in the next, just as an ordinary nephridium does; and in Plutellus, where the nephridium does not pass through a septum, but lies wholly in one somite, so does the oviduct.

The modification which the nephridium undergoes to form a genital duct consists either in—

(*a*) A fusion of a series of nephridia; or

(*b*) A disappearance of a part of the nephridium ; or

(*c*) A shifting of the position of the pore.

In the case of the male duct each of these modifications is exhibited. In the somites, in which the ciliated rosettes are, the external extremity of the nephridium has disappeared ; in the somites carrying the male pore, the funnel region of the nephridium is absent, whilst in the intervening somites both these regions have aborted, and a fusion of these various parts has taken place to form the more or less elongated duct. We are not warranted in supposing that these changes have actually occurred in the development of these forms ; it is possible that the appearance exhibited is the retention of a condition such as is seen in Hatschek's Polygordius larva.

In the oviduct the intermediate portion between the funnel and the pore has become very much shortened and widened.

Again, in the case of the spermatheca, the greater part of the organ has aborted, whilst the remnant has swollen to form a sac in most cases, though in those Perichætæ, where the coiled appendix is present, this may perhaps represent the tubular, whilst the sac represents the vesicular, portion of the original nephridium. But it seems to me that there is much greater doubt and difficulty in the case of the spermatheca. [For in Microchæta, as will be seen, there are six or eight spermathecæ to a segment, each being separate and opening in a transverse line, more like the small sacs surrounding the spermatheca in Perichæta aspergillum.]

It is possible that there is a great distinction between the vesicular portion and the rest of the structure ; the former may be merely the invagination of the integument to meet the glandular secretory tube which itself has had a very different origin, as suggested by Lang in his researches on Planarians. The spermatheca would correspond then with this non-essential vesicular portion of the normal nephridium.

PART II.

Descriptions of New or Little-Known Earthworms.

I. *Microchæta Rappi*, Beddard (*Lumbricus microchetus*, Rapp).

Introduction.—Last year Prof. Ray Lankester received from Dr. George Romanes, F.R.S., two very large Earthworms which had been sent to him from South Africa, by Dr. J. W. Stroud, of Port Elizabeth, Algoa Bay. He kindly placed them in my hands for dissection. They were living when they arrived, so that I was able to make some observations in the fresh state. They were then placed in chromic acid, and afterwards in 30 per cent. spirit, being ultimately preserved for sections in strong spirit. The examination of their external characters proved them to belong to a species which had been described by Mr. Beddard in the autumn, in a paper read before the Zoological Society.

a. External Anatomy.—The worms measured about three feet six inches in length, and averaged about three quarters of an inch in width, though wider anteriorly. A life-sized, and naturally-coloured drawing was made by Miss Stone. The surface of the body is in colour, a beautiful iridescent, greenish brown dorsally and laterally, whilst ventrally it is of a pink tint. The clitellum is deep green, with a bright pink orange under-surface. The anterior and posterior extremities are very obtuse, whilst the body is nearly cylindrical, not being much flattened.

The prostomium is a very small lobe and not embedded in the first or buccal somite.

The somites themselves are not by any means as distinct as in Lumbricus, but each consists of a number of annuli, so that, from the exterior it is difficult to limit the anterior somites. The number of annuli to a somite in this region varies; but in the clitellum there are three annuli to a somite, and posteriorly

three or four, one of the annuli being sometimes subdivided. It was by tracing the nephridiopores that it was possible to ascertain the limits of the somites. The setæ are much too minute to be of any assistance, as they are extremely difficult to see, whilst the nephridiopores are very evident. By this means, and subsequent dissection, I have drawn up the following table. I count the first nephridiopore as being in the first annulus of somite II.

Somite	I	consists of	3	annuli (exclusive of the prostomium).
„	II	„	7	„
„	III	„	6	„
„	IV	„	7	„
„	V	„	6	„
„	VI	„	6	„
„	VII	„	6	„
„	VIII	„	4	„
„	IX	„	3	„
„	X	„	3	„
and in all subsequent somites			3	„

Whether these numbers are constant for every individual I cannot say.

There is a great thickening of the body in somites IV, V, VI, VII, due to the thickness of the muscular layers of the body wall, more especially to that of the longitudinal muscles.

The clitellum is very noticeable on account of its green colour; it is further forwards than in Lumbricus. It extends over the somites XIII to XXV inclusive. (Pl. XV, fig. 1). Its boundaries, however, are not very distinct, since both anteriorly and posteriorly it merges into the neighbouring somites; it does not extend completely round the body, as is the case in Perichæta, but resembles that of Lumbricus. In histological structure it differs somewhat from the latter Earthworm, as will be seen below.

Setæ.—The setæ are arranged, as in Lumbricus, in four couples in each somite, the two setæ forming a couple being very close together. One pair of couples is quite lateral (*s. l.*) the other pair latero-ventral (*s. v.*). (Plate XV, fig. 1 and 1*b*).

The setæ are very minute, whence the name of the genus

given by Mr. Beddard. The ventral setæ in the posterior region of the body are ·52 mm. long, and have a strongly curved embedded portion whilst the free end is only slightly curved; there is the usual thickening in the middle region (Pl. XVI, fig. 29). The lateral setæ are three quarters this size, ·4 mm. long, and I found it impossible to extract them: it is only by means of sections or by teasing up a piece of body wall that one can see them, although by means of a lens their pits can be seen superficially.

In the anterior region the ventral setæ are rather longer, and differ in shape: the thickened region, usually about the middle in the ordinary setæ, is here just below the free end, giving the appearance of a spear-head to the seta. (Pl. XVI, fig. 28).

The free ends in many cases were much worn.

External Apertures.—The mouth is nearly terminal, being overlapped by the small prostomium; it is large and circular, and the surrounding "buccal" somite seems to be able to be used as a sucker, from what I saw of it when the animal was alive.

The anus is a sub-terminal, horizontal slit.

The dorsal pores are absent; I could see none either by means of a lens, or in a series of transverse sections through the posterior region of the body.

The nephridiopores are very evident (Pl. XV, fig. 1). Each appears as a longitudinal depression in a line with the lateral setæ. Since the width of a pore is as great as the space between the two setæ forming the lateral couple, it is difficult to say whether they are in a line with the upper or lower seta of a couple. Thus they differ in position from those in Lumbricus, where they are in a line with the ventral setæ.

Each nephridiopore (*ne. o.*) occupies the anterior edge of the first annulus of a somite, and it was by counting the number of annuli between the pores that I was able to ascertain the number of annuli that go to make up a somite.

The first nephridiopore is in the fourth annulus, and as there is never a nephridium in the first somite I regard this annulus as the commencement of the second somite. Thus somite

I consists of three annuli. The second pore is placed in the thirteenth annulus, thus giving seven annuli to somite II, and so on.

The oviducal pore was not to be found, nor could I find an oviduct internally; but since the ovary is in somite XIII, as in Lumbricus, the oviduct is presumed to be in that neighbourhood.

The spermathecal pores are very numerous and minute; it was only after dissection that I found where they are situated, since the spermathecæ have the unusual position of being behind the ovary; they open on the anterior edge of somites XII, XIII, XIV, XV, outside, i. e. dorsad, of the lateral setæ, and vary from one to four in each case. (Pl. XV, fig. 1, *spth. p.*)

The sperm-pores are not evident superficially, as there are no papillæ or other marks in their neighbourhood; but by tracing down the sperm duct I found it to end in somite XIX, (fig. 1, *mp.*). Thus the worm resembles the large forms from America, Titanus and Anteus, in being Intraclitellian.

Although the worm was mature I could find no capsulogenous glands. I hope to return to the subject of capsulogenous glands in a subsequent paper.

The Body Wall.—The general structure and arrangement of the various layers is the same as in Lumbricus, but there are various small details in which a section through the body wall of the one differs from that through the other, both in the clitellar region and in other parts of the body. (Plate XVI *bis*, figs. 39 and 40.)

The ordinary epidermis consists of the usual elements, viz. columnar cells (*col.*) and goblet cells (*gb.*)

The columnar cells appear to be more squeezed together, if possible, towards their inner ends, than is the case in Lumbricus; the nuclei of these cells are rather nearer the surface than in that form.

The cuticle (*cu.*) is traversed by striæ in two directions, and shows the numerous pores from the goblet cells, each at the junction of two striæ. (Pl. XV, fig. 1 *a.*)

The goblet cells are rather more numerous than in

Lumbricus; they are filled with the same large globular granules, but the nucleus, instead of being near the base of the cell, is sometimes in the centre of the cell, rarely so close to the base as in Lumbricus. Some of these cells seem to be emptied of their granules, leaving a network of protoplasm (fig. 40, *b*). Frequently a goblet cell appears to have more than one nucleus, but a faint line can generally be seen (*a*), and I take it that the goblet cells being very closely packed, a part of a neighbouring cell and nucleus is included in the section. The basement membrane is fairly thick (*bm.*).

The circular muscular layer (*musc. circ.*) is very much thicker compared to the epidermis than in Lumbricus, nor are the strands of muscle so closely packed nor so regularly circular as in that worm. In Microchæta some of this layer appears to be rather oblique, and the muscle-fibres to be grouped in strands, and separated more frequently by connective tissue than is the case with Lumbricus. Between the circular and the longitudinal layers of muscle is a fairly thick layer of connective tissue (*ct.*)

The longitudinal muscles (*mus. lg.*) present the chief point of difference between the structure of the body wall of Microchæta and of Lumbricus. In the latter form there are groups of muscles, where the strands are arranged on each side of a radial piece of connective tissue, each group appearing more or less separate from the neighbouring group and thus having a bipinnate arrangement. But in Microchæta we have no such grouping. The muscle-strands are all packed together in connective tissue. The section of the strands is circular, or elliptical—not linear as in Lumbricus—and we have two or three strands grouped together and surrounded by connective tissue; so that this layer, when the section is stained in Borax carmine, appears as a number of more deeply stained, more or less circular masses, each in a setting of connective tissue. A similar difference in the breaking up of the longitudinal muscular layer is found to obtain amongst various genera and subgenera of Sipunculid Gephyreans.

The whole depth of this layer is very great, being five or six

times that of the epidermis; both muscular layers are well supplied with blood-vessels, which, however, do not penetrate the epidermis.

Embedded in this layer are, here and there, more deeply-stained masses of cells, with well-marked nucleus in each (*mlr. gl.*); from these masses a duct may be traced towards the surface, but when it reaches the epidermis this duct has become very fine, so that, though doubtless it passes between the cells, it is very difficult to follow. I regard these as multicellular glands, which open to the surface.

In the region of the body between somites VI and IX, the longitudinal muscular layer is enormously thick—that is, in the region of the strong septa—giving a very much greater rigidity and strength to the animal here than elsewhere.

Beneath the longitudinal layer is another layer of fibrous connective tissue, thicker than the layer outside the longitudinal muscles, but similar to it, and continuous with it by the intermediation of the tissue around the muscle-strands.

The parietal portion of the cœlomic epithelium (*cœ.ep.pa.*) consists, as in Lumbricus, of flat cells whose nuclei are evident in sections.

The Clitellum.—In the clitellum of Lumbricus we have the three following chief constituents Claparède (11), and Mojsisovics (50):—

1. Ordinary columnar cells, but usually shorter than in the other regions of the body.
2. Narrow, elongated cells, with globular granules similar to those found in the goblet cells of other parts of the epidermis; but these cells are much longer than the columnar cells, being nearly three times their length.
3. Very long cells, swollen at their inner end, and containing densely packed minute granules; these are the club-shaped cells. They are five or six times the length of the columnar cells.

These all have branched bases, and in the two last varieties the nucleus is found near the base of the cell.

In Microchæta (Pl. XVI *bis*, fig. 41) we have a fourth

element in the presence of goblet cells similar to those found in the other regions of the epidermis. Another difference presents itself in that the cells No. 2 are not so long as, but much narrower than, those in Lumbricus.

Another point of interest is a network of connective tissue at the base of the epidermic cells. This loose network (*nt.*) is continuous with radial strands which separate the groups of club-shaped cells, and are themselves continuous with the connective tissue between the muscle strands. Accompanying the connective tissue and ramifying between the groups of cells are numerous capillary blood-vessels.

c. INTERNAL ANATOMY.—The points which strike one most on opening the worm are, the immense thickness of the anterior septa, even stronger than those figured by Perrier for Titanus and other large worms; the distance of these septa from one another; their funnel-like shape and freedom from any overlapping; the large size and curious shape of the nephridia, with their large vesicle and pinkish rosette of tubules (these are shown in Pl. XV, fig. 2); and, lastly, the rich cherry-red colour of the cœlomic epithelium which clothes the intestine (instead of the yellow colour of this tissue in Lumbricus).

The Septa.—The first septum is between the somites that I consider as III and IV, since there are two pairs of nephridia in front of it, the first pair belonging to the second somite. It is very thick, and its central portion is carried some distance back, though not so far as the next septum, closely adherent to the œsophagus, whilst peripherally it spreads out, and is fixed to the body wall by means of its own substance and also by means of muscle-strands (*mb*) which pass from its posterior side outwards and backwards to the body wall. The second, fourth, and fifth septa are similar to this. Perrier has suggested that the use of these strong septa is to give firmness as well as strength to this region of the body for the purpose of burrowing. The third septum, that immediately in front of the gizzard, is very much thinner, in fact not much stronger than the posterior septa; this probably allows the gizzard some freedom of movement forwards and backwards. The sixth, seventh, and eighth

septa are slightly thinner than this third one, whilst the septa subsequent to these are much weaker.

The Alimentary Tract.—The digestive tube consists of (*a*) Buccal region, (*b*) Pharynx, (*c*) Œsophagus, (*d*) Gizzard, (*e*) tubular intestine with gland, (*f*) sacculated intestine, and (*g*) Rectum (Pl. XV, figs. 2 and 3).

(*a*) The Buccal region (*B* in the figure) immediately follows the mouth; it is a short, thin-walled region, and seems to be slightly protrusible, thus bringing the muscular pharynx into direct contact with the food, which can then be clasped by this organ. This region seems frequently to be omitted in descriptions of Earthworms; it is certainly very limited in extent, but seems, according to Perrier (14), to be pretty constant, and its posterior limit is marked by the circumpharyngeal nerve commissure. In Microchæta it only extends to the third annulus, i. e. through one somite.

(*b*) The Pharynx (*Ph.*) then follows, and, as in other worms, is a very muscular organ, and besides its intrinsic muscles is held to the body wall and first septum by numerous radiating muscles (*r.m.*), some of which are very thick. The pharynx does not quite reach the first septum, and thus only occupies somite II and part of somite III. As a rule the pharynx extends through four or five somites, but here the somites, although only two in number, are very long, and the pharynx has an extent of nearly one inch. The limit of the pharynx is often incorrectly stated, e. g. that of Lumbricus is put down as reaching to somite VII whilst really it extends only to the end of somite V; this error is due to the infundibulate shape of the septa. As for the vexed question of glands in the pharynx, which Perrier seems inclined to consider present, I could find none, either by dissection or by means of transverse sections; Claparède figures none in his paper on the histology of Lumbricus (11).

(*c*) The Œsophagus (*Oe.*) commences just in front of the first septum, and passes through the somites IV and V. The wall is thin, and close in front of the septa through which it

passes there is a mass of brown pigment. I expected to find glands of some sort here, but sections showed that the pigment was in the connective tissue round the œsophagus; the wall internally is raised into numerous circular and longitudinal villi (Pl. XVI, fig. 27).

(*d*) The Gizzard (*G.*) then follows in somite VI. It thus lies very much further forward than in Lumbricus, though in other forms it has nearly as forward a position as in Microchæta. Perrier has noticed that this forward position of the gizzard accompanies the forward position of the clitellum in Post-clitellian and Intraclitellian worms.

The gizzard has the same appearance as in Lumbricus, being rather shiny, vascular, and hard; but in this case there is a constriction near its hinder end, so that we have a large anterior portion, which alone has the characteristic chitinous lining secreted by the epithelium, and a smaller posterior portion which is simply muscular; thus the anterior division is the functional crushing organ.

(*e*) The tubular intestine (*int. t.*) leads from the gizzard to the sacculated intestine in somite XIII. It is cylindrical, has a diameter about two thirds that of the œsophagus, has a fairly thick muscular wall, and its internal epithelium is raised into longitudinal ridges, as is the case in that of the œsophagus. In somite IX there is a hemispherical swelling (*gl. int.*) on each side, the intestinal gland, which in the fresh state is red in colour owing to the highly vascular character of the wall. These glands are merely saccular enlargements of the lumen of the intestine.

In section they have somewhat the appearance of Claparède's figure of the œsophageal gland of Lumbricus. The wall consists of a number of tubular glands radiating away from the intestine; each gland consists of a lumen surrounded by squarish, granular cells, and between each of these closely-packed tubes is a broad space, continuous with the vascular network on the surface of the whole gland, and enlarging as it nears the lumen of the intestine. I tested for carbonate of lime in a portion of the gland and was successful in obtaining an effervescence proving the exist-

ence of this substance, crystals of which could be seen in the section; so that in structure and contents it agrees with the œsophageal gland of Lumbricus.

Such "intestinal glands" have been described as occurring in Urochæta (28), where Perrier calls them "glandes de Morren," and considers them as having the same function as the calciferous or œsophageal glands of Lumbricus. But while in this latter form they are in front of the gizzard, in Urochæta and in Microchæta they are posterior to it.

(*f*) The Sacculated Intestine (*int. s.*).—The tubular intestine gradually widens in somite XII, till in the next somite it has increased to about three times its previous diameter, and in the succeeding somites retains this diameter, being, however, constricted as it passes through the septa; this gives this region a sacculated appearance. It has very thin walls, which, in the fresh state, have a cherry-red colour, due partly to the vascular network on its wall, and partly to the brown red granules in the large cells of cœlomic epithelium covering the wall—similar cells to those on the intestine of Lumbricus.

This region differs from the tubular intestine in the possession of a typhlosole (*ty.*), which commences in somite XIII, and is continued nearly to the posterior third of the body. The typhlosole is cylindrical, and very large, nearly filling the lumen of the intestine; the brown-red vesicular cœlomic cells are continued into it, and fill it, being traversed by the irregular typhlosolar trunk.

A section across the wall of the intestine (Pl. XVI *bis*, fig. 42) shows a condition very similar to that in Lumbricus. The intestinal epithelium (*int. ep.*) is thrown into ridges, and the cells are longer on the typhlosole than on the outer wall. The majority of the cells are ciliated, and between them are some cells with granular contents which are stained by borax carmine; these are probably glandular (*gl. c.*) digestive cells.

The muscular layers are more largely represented than in Lumbricus; and between the circular muscles and the epithelium are the great blood sinuses, which form a close network on the wall. The large cœlomic epithelium cells have the same

club shape with coloured granular contents as in Lumbricus (*c. e. vis.*)

(*g*) The Rectum differs from the sacculated intestine chiefly in the absence of the typhlosole, and the absence of the coloured granules in cœlomic epithelium.

The main points of difference then between the alimentary tract in Microchæta and in Lumbricus, is in the absence in the former of glands on the œsophagus (a very usual difference); the absence of a proventriculus; the position of the gizzard in somite VI, instead of in somites XVII, XVIII, and in the presence of an intestinal gland.

It is noteworthy that the septum immediately in front of the gizzard is very thin; similarly in Lumbricus, the septum between somites XVII and XVIII, occupied by the gizzard, is nearly deficient; evidently this is related to the necessity or habit of moving the gizzard during feeding.

The Genital Organs.—The genital organs were all well developed in the two worms opened, and are formed in the same type as in Lumbricus; and, as in that form, there is no penis, or prostate. The most interesting organs are the spermathecæ, both in their position, their number, and their small size. (Pl. XV, fig. 4.)

The genital organs consist of the following:

A. Male: (*a*) Seminal reservoirs; (*b*) testis; (*c*) sperm ducts and ciliated rosettes.

B. Female: (*a*) Ovaries; (*b*) spermathecæ.

C. Certain structures of unknown functions in somite XII, which, being in the genital region, I shall describe here, though whether they have or have not any relation to the generative organs, I do not know.

A. Male Organs.—*a.* The seminal reservoirs, or "testicular sacs" (*sem. res.*), are four in number; a pair of nearly spherical, light brownish sacs in somite X, and a pair in somite XI. They are placed, as usual, close to the intestine, which they partly overlap; but they are not so irregular as in Lumbricus, and are much firmer. In section (Pl. XVI, fig. 11) they are seen to be made up of trabecula of connective tissue, traversed by

blood-vessels; and in the spaces between the trabeculæ are the developing spermatozoa. This is the same structure as in Lumbricus, but much more compact, and the spermatospheres are fewer in number.

Each sac is united to its fellow of the opposite side, but the anterior pair remain quite distinct from the posterior pair. We may take the sac in somite x for further examination. Each seminal reservoir is constricted as it passes forward through the septum into somite IX into a very narrow neck; in this somite it swells out again into a globular sac, which lies in the posterior half of the somite; this contains the ciliated rosette of the sperm duct, and so it may be called the "rosette-sac" (*r. s.*). This latter sac unites across the middle line with its fellow of the opposite side, so that the cavity of the two seminal reservoirs of somite x are continuous in somite IX. The sacs in somite XI have the same arrangement.

b. Testis.—From the rosette-sac, close to the middle line, there springs a cylindrical horn-like process (*t. pr.*), which runs outwards and forwards, and abuts by an obtuse, rounded end against the anterior septum of somite IX; there are two of these in somite IX, one from each of the rosette-sacs. This prolongation is hollow, and its wall consists of a thick layer of connective tissue; there springs from a line along its outer side a mass of cells with large nuclei, amongst which blood-vessels run; this mass more or less fills the lumen, and is a testis (Pl. XVI, figs. 12, 13). The cells are like the sperm mother-cells of testis of Lumbricus. This arrangement is repeated for the pair of rosette sacs in somite x. Thus there are two pairs of seminal reservoirs in somites x, XI; two pairs of rosette-sacs in somites IX, x, and two pairs of the above prolongations with enclosed testes in somites IX, x. The compactness of the seminal reservoirs, their more regular shape, and the fewer masses of spermatozoa suggest that in this worm the production of spermatozoa is not periodical, as in Lumbricus, but more continuous, whilst the "testes" are conspicuous, though enveloped in a cæcum of the rosette-sac. The testis has the same appearance in a section through a mature Lumbricus, as

in Microchæta, but it is enclosed only in the thin-walled seminal reservoirs, whilst in the latter it is enclosed in a special cæcum of the seminal reservoir, which possesses a strong wall.

c. The Sperm Ducts and Ciliated Rosettes.—Each of the four ciliated rosettes (*c. r.*) lies, as stated above, in a sac; two in somite IX, two in somite X. From each rosette there leads a narrow sperm duct (*sp. d.*), which immediately passes through the septum, and then backwards and outwards till it reaches the next septum, beyond which it is continued directly backwards, closely adherent to the body wall, and just within the line of the nephridiopores, to the commencement of somite XIV. Here it joins the other duct of the same side, and the two pass on as one duct to their external pore in the somite XIX. The separation from one another of the two ducts of one side for so many somites is perhaps noteworthy. There are no accessory copulatory organs.

B. Female Organs.—(*a*) Ovary.—Lying on the anterior septum of somite XIII, close beside the intestine, on each side, is a dark hemispherical mass of cells (*O.*) (figs. 2, 4), appearing, even to the naked eye, to be made of a number of lobules; it is fairly large, being about one eighth inch along its base. These are the ovaries, and microscopic study shows that each is made up of a number of lobules, which contain masses of ova (Pl. XVI, fig. 14). I could see no gradation in size amongst the ova of any given lobe, corresponding to a difference in age of the ova, such as we find in the ovary of Lumbricus. Each ovum, consisting of a granular protoplasm with well-marked nucleus and nucleolus, is surrounded by cœlomic epithelial cells.

Thus the shape of the ovary differs a great deal from what obtains in Lumbricus, and resembles the ovary in Plutellus and Pontodrilus; being as it were made up of a number of Lumbricus' ovaries, each without the characteristic "tail." It may be that here the ova were not far enough advanced, that they were too young, to show this "tail;" for in young ovaries of Lumbricus the tail is not very pronounced; in the case of Microchæta we should then get a tail to each lobule.

Oviduct.—I could not identify any structure as the oviduct.

b. The Spermathecæ.—In somites XII, XIII, XIV, and XV, close to the anterior septa, and immediately outside the nephridia, are a number of small, whitish, horseshoe-shaped bodies (*spth.*) (Pl. XV, figs. 2, 4). These I found to contain spermatozoa, of the same shape as in Lumbricus, and I therefore conclude that these curious organs are spermathecæ (Pl. XVI, figs. 9, 10). Each consists of a sac lined by columnar cells, and surrounded by two sets of circular muscles at right angles to each other. The number varies, both in the somites, and on each side of the same somite; the average is three on each side, though in somite XV there were four on one side and three on the other, whilst in somite XII, only two were present on each side. They are not all the same shape, some being U-shaped, and some being ω-shaped. Each of these has a separate opening to the exterior, those of one somite all being in a line at the anterior edge of that somite; their external pores can be seen only by cutting through the body wall along this line, where their white colour shows where they open, or by means of sections through this region. Their pores are mostly outside, that is dorsad, of the nephridiopores, though in one or two cases the innermost spermatheca is in a line with the nephridiopores. Their position behind the other genital organs is very peculiar. The only other worm of which I can find a description of such a condition is Eudrilus decipiens, E. P. (14), which has spermathecæ in the next somite behind the testicular sacs; but none has so large a number, that is, twenty-two to twenty-four; nor of so small a size, viz. about one twelfth of an inch across the ends of the horseshoe. Their small size is perhaps related to their large number, but the position of some of them behind the ovary is certainly very striking.

c. Certain doubtful Structures.—(*a*) On the septum between somites XII and XIII, on the opposite side of the septum to that on which the ovary lies, that is to say, in somite XII, is a rosette-shaped organ (*x*) (figs. 2, 4, and 7). When

examined by means of a lens it looks somewhat like the ciliated rosette of the sperm duct, but is much less folded. It is ciliated along its edges and on the surface; the appearance of the surface is of a number of more or less hexagonal cells placed close together; these are the ends of short columnar cells ciliated along their free surface (*xp.*, Pl. XVI, fig. 14). The rosette is fixed to the septum at about its centre, and appeared to be the internal funnel of some duct, but this duct I was unable to find by dissection, owing to the muscularity of the septum. This rosette resembles the figure of the "fimbriated organ" described by Beddard in Pleurochæta (Megascolex) (36), the function of which he did not ascertain. In Microchæta it may be the funnel-shaped internal opening of a very delicate duct to convey to the exterior the products of a gland (*y*), which I will now describe.

(*b*) On the anterior septum of somite XII is a glandular-looking structure (*y*), whose function I do not know. It consists of a dense mass of rounded cells arranged in a band, which is bent upon itself several times (Pl. XVI, fig. 8), the folds being close to one another. As, unfortunately, I did not observe it till after the animal had been in spirit, I am unable to say what its appearance is when fresh; one might imagine it to be an ovary, whose duct is the organ just described, but its cells are not large enough, nor have they the characteristic structure of egg-cells. Has it something to do in the formation of the egg capsule, or is it connected in any way with copulation? I cannot say. But Megascolex (Pleurochæta of Beddard) (30) and Acanthodrilus, E. P. (14), have, similarly, organs in this region whose function is doubtful.

The Vascular System.—The blood system consists of the following longitudinal vessels, (*a*) Dorsal trunk, (*b*) a Ventral trunk, (*c*) a Typhlosolar trunk, (*d*) Lateral trunks, as well as lateral loops, amongst which are some strong commissural vessels or "lateral hearts" (Pl. XV, figs. 5, 6). There is no subneural trunk.

(*a*) The Dorsal Trunk.—Lying on the top of the intestine is a thin-walled tube, constant in diameter, which is about one

tenth of an inch throughout the greater part of the body, but which is modified anteriorly; this is the dorsal trunk (*D.*). In many forms, e.g. Urochæta and Titanus, the dorsal vessel is, in some parts at least, ampullate, but in Microchæta this is not the case. This thin-walled tube may be traced forwards, till in somite XIII it becomes somewhat narrower, and continues with this less diameter till, in somite IX, it enlarges again and becomes more muscular, whilst in somite VIII is a very noticeable heart-shaped swelling (*d. sw.*). At first sight it appears to be merely an enlargement of the dorsal trunk with strong walls, but on opening it we find it contains a double lumen (Pl. XVI, figs. 15, 16). In fact the dorsal trunk has here divided into two more or less parallel vessels, lying quite close together, and having thick muscular walls; each of these vessels commences posteriorly with a narrow lumen, but as it passes forwards gradually enlarges and bulges anteriorly, then very suddenly narrows again and unites with its fellow to form a single dorsal trunk, as in the posterior part of the somite. This single portion passes through the anterior septum into somite VII, becoming much narrower, and then divides into two vessels parallel and close to one another, each of a little less diameter than the single portion from which it springs; these two again unite just behind the septum and pass through as a single trunk. The same thing happens in somites VI, V, IV (*dd.*, fig. 5), the dorsal trunk getting narrower and narrower. Thus we have, besides the enlarged double portion in somite VIII, a narrow double portion of the dorsal trunk in somites VII, VI, V, IV. This splitting of the dorsal trunk is described by Beddard in Pleurochæta (36) and in Acanthodrilus sp. (40). In this paper he also describes the arrangement in Microchæta. In the somite III and onwards, anteriorly, the vessel lies on the pharynx, and it remains single, but divides just behind the cerebral ganglia into two vessels, one on each side, which pass downwards (*b.r.*), I am uncertain whether directly into the ventral trunk, or if they break up into a network in the pharynx, and thus are continued into the ventral trunk.

Branches from the Dorsal Trunk.—In the region of the

sacculated intestine, i. e., behind somite XII, two vessels communicate with the dorsal trunk in each somite. One of these is the "intestinal branch" (*d. int.*), which leaves the trunk near the anterior limit of the somite and passes round the wall of the intestine, giving off numerous branches fore and aft, forming a network as in Urochæta, on the intestinal wall. This vessel does not unite with the ventral trunk. Just in front of its exit from the dorsal trunk is a small valve, directing the blood, which passes from behind forwards in the dorsal trunk, into the intestinal vessel. (Pl. XVI, fig. 17, *va. i.*)

The second vessel is the "septal branch" (*d. spt.*) which comes off close to the posterior septum of the somite. It passes along this septum, giving off branches to it, and then reaches the body wall, where it joins one or more longitudinal vessels, which give off right and left branches and thus form a network on the wall (*l. w.*). A vessel from the nephridium joins this septal branch. Just behind the entrance of this septal vessel into the dorsal trunk is a small valve (fig. 17, *va.*), which prevents the blood, on contraction of the latter vessel, from passing into the former. The septal vessel therefore brings aerated blood into the dorsal trunk, whilst the intestinal branch passes this blood on to the wall of the intestine.

These same two pairs of vessels are found in somite XII, whilst in somite XI only the septal vessel is present.

In somites X, XI a branch from the dorsal trunk goes to the seminal reservoirs.

In somites X, IX, VIII, VII, VI, V, and IV, the only vessels which leave the dorsal trunk are the "commissural vessels" (*com.*), which pass round the alimentary tract and enter the ventral trunk. Of these, those in somites X, IX, VIII, VII, and VI are large and moniliform, and may be specially termed "lateral hearts" (*com'*). The three posterior pairs are very large, but the same description applies to each.

Each "lateral heart" leaves the dorsal trunk close to the posterior septum of the somite, and in the somites where the dorsal trunk divides into two, it arises from the undivided portion, and its exit is guarded by a valve (*va. c.*, Pl. XVI, fig.

16). The proximal portion of the lateral heart is narrow, but the vessel soon swells into a globular form; the vessel presents a series of such dilatations. The moniliform appearance is due to circular muscles placed at certain distances along the vessel (fig. 18). These five pairs are contractile: that is why they may be called "lateral hearts," whilst the other two pairs of commissural vessels are non-contractile.

A few very small vessels leave these "hearts" and go to the posterior septum, and another larger one has a similar course from near the ventral end of the "hearts;" but in these somites there are no vessels from the dorsal trunk to the alimentary tract.

In somites V and IV the commissural vessels (*com.*) are not moniliform and are much narrower than the "hearts;" like them they leave the dorsal trunk at the undivided portion. Shortly after leaving this trunk, each gives off a small septal branch (*com. spt.*) to the posterior septum of its somite; whilst in addition they give off a vessel to the œsophagus (*com. al.*).

In somite III the dorsal trunk has become very narrow and remains single; just in front of the first septum it gives off a branch to the wall of the pharynx on each side (*ph. v.*), which after giving off a branch to the septum (*spt.*) breaks up amongst the muscles of the pharyngeal wall into a network (*ph. nw.*) with which the lateral trunks (*L.*) are connected.

About half-way along the pharynx, that is, in somite II, a second pair of branches is given off to the pharyngeal wall (*ph. v'.*), which also helps in the formation of the network just mentioned.

A third pair of branches (*br.*) to the pharynx occurs by the division of the now very delicate dorsal trunk into two vessels, just behind the cerebral ganglia. Each of these passes downwards, close behind the circumpharyngeal nerve commissure, either to enter the ventral trunk directly, or to break up into the network from which both ventral and lateral trunks take origin. The existence of the three pairs of branches from the dorsal trunk in front of the first septum seems to confirm the idea, that this region is formed of three somites.

Thus, in the dorsal trunk, the greatest contractile region is in somites VIII and IX, whilst the only direct communication between it and the ventral trunk is by means of the seven pairs of "commissural vessels," of which the posterior five pairs are contractile.

(*b*) The Ventral Trunk.—This longitudinal median vessel commences anteriorly, either by the union of the two circum-pharyngeal branches (*br.*) of the dorsal trunk, or in a network formed by their subdivision. It passes directly backwards (*V.*) lying rather nearer the nerve-cord than in Lumbricus, and has much more muscular walls than the dorsal trunk has: after receiving various branches, it enlarges in somite X, and remains much the same size throughout the body.

In somites IV, V, VI, VII, VIII, IX and X it receives the ventral ends of the commissural vessels, near the posterior boundary of the somite in each case (*com. com'.*).

In somites X and XI branches leave it, to go to the seminal reservoirs (*v. t.*), and in all of the somites a pair of vessels passes from the ventral trunk to the septum (*v. spt.*), and supply a branch to the nephridium. Behind somite XII three or four small vessels in each somite from the wall of the intestine (*v. int.*) enter the ventral trunk.

(*c*) The Lateral Trunks.—In the anterior somites of the body a longitudinal trunk is seen on each side (*L.*), lying close to the alimentary tract and rather ventrad of it, passing below the sides of the gizzard. These "lateral longitudinal" trunks have no direct communication either with the dorsal, or with the ventral trunk, thus differing from the pair of lateral longitudinal trunks in Lumbricus, which arise as branches from the dorsal trunk. Each lateral trunk rises from a vascular network on the pharynx (*nw. ph.*) and ends posteriorly in a network on the intestinal gland of somite IX (*nw. int.*). In each somite, through which it passes, it receives a small vessel from the posterior septum (*l. spt.*), and a vessel from the alimentary tract (*l. al.*); this is usually small, but in somite VIII a large vessel from the gizzard enters the lateral trunk.

The direction of the blood in these lateral trunks is difficult

to determine, so that whether the blood enters it, or leaves it by the branches mentioned, is uncertain; but it seems probable, from the consideration of the arrangement of these branches, and the supply of the neighbouring organs by the dorsal trunk, that it receives blood from the septa and from the alimentary tract, carrying some of it forwards to the pharynx.

(*d*) The Typhlosolar Trunk.—This is an ill-defined vessel, as seen in sections, lying in the typhlosole, and commencing in somite XIII (*T.*). It communicates, frequently, in each somite with the dorsal trunk, and receives a branch from the wall of the intestine (*t. int.*) in each somite, near the posterior septum. This intestinal vessel receives anterior and posterior branches, which help to form the capillary network on the wall of the intestine, contributed to also by similar fore-and-aft branches from the intestinal vessel of the dorsal trunk. These intestinal vessels of the typhlosolar trunk are veins, pouring blood into the typhlosole, whence it passes into the dorsal trunk by small vertical vessels.

The Course of the Blood (figs. 17, 19, 20).—Perrier has described minutely the course of the blood in Urochæta (28), and probably the main points are the same for most Earthworms, but in Microchæta the absence of a subneural trunk causes some variation. As is well known, the blood in the dorsal trunk passes from behind forwards, as it also does in the typhlosolar trunk.

The blood is directed out of the dorsal trunk, through the intestinal branches, by means of the valves placed at the exits of these. In the posterior region the following is what appears to be the course: by the intestinal vessels the blood is carried to the network on the wall of the intestine; from this network the blood passes by means of other intestinal vessels into the typhlosolar trunk and thence forwards.

In this region, also, the blood is passing backwards in the very contractile ventral trunk, from which it passes by the septal branches to the septa, nephridia, and body wall; from the various networks on these structures the blood is collected by branches, which, in each somite, enter the dorsal

trunk, and on arriving there it is sent forwards (Pl. XVI, fig. 20).

The blood thus poured into the dorsal trunk has been aerated on the body wall and purified in the network on the nephridia, and is then sent forwards, some of it passing to the intestine, where it gathers nutritive material, which it pours into the typhlosolar trunk, and thence back into the dorsal trunk.

With regard to the course of the blood in the lateral trunks, it is difficult to be certain whether it is backwards or forwards; whether they collect blood from the network on the pharynx, from the œsophagus and septa of this region, and pour it into the network on the wall of the intestinal gland in somite IX; or, on the other hand, whether they collect the blood from this gland, and pass it forwards to the pharynx, receiving fresh supplies from the alimentary tract on its way, as well as aerated blood from the body wall, &c.

It is this latter alternative that Perrier adopts in the case of Urochæta, where the lateral (or "intestino-tegumentary") trunks have a similar disposition; so it is probably here. Thus these lateral trunks will resemble the dorsal trunk, and the typhlosolar trunk, in the direction in which the blood is going.

Microchæta agrees with Pontodrilus and differs from most other Earthworms in the absence of a subneural trunk.

The Nephridia.—One of the most noticeable features in Microchæta is the size and shape of the nephridia (Pl. XV, fig. 2). The apertures have already been mentioned as being in a line with the lateral setæ, in the usual position, in the anterior region of the somites. The nephridia themselves lie close to the anterior septa of the somites, and each is very like Perrier's figure of the first nephridium of Urochæta—his "glande à mucosité."

Each nephridium consists of three parts (Pl. XVI, fig. 21):

(1) A large vesicular portion, communicating with the exterior on the one hand, and on the other with (2) a rosette of tubules, from which a branch passes through the septum to (3) the internal funnel.

(1) The vesicle (*ne. v.*) (fig. 21, Pl. XVI) is a very large,

conspicuous sac, subtransparent when fresh, and contrasting with the pinkish mass of tubules. Muscles traverse it in all directions; and round the external pore (*ne. o.*) they are concentrated into a set of circular muscles, acting as a sphincter, outside which are radial muscles. Its cavity is lined by columnar cells, between which and the muscles is some connective tissue with a poor supply of blood-vessels.

In one of the ordinary nephridia (for the shape of the vesicle varies somewhat in various regions, figs. 21, 25, 26) the external pore is not situated at one end of the vesicle, but very much nearer the rosette of tubules; whilst the vesicle is prolonged outwards, more correctly dorsally, and ends in an obtuse, blind end (*c. v.*), near the mid-dorsal line above the alimentary tract. At the opposite end the vesicle is rapidly constricted, and it is here that the rosette of tubules is attached; these are well supplied with blood-vessels which give the rosette a pink appearance when fresh.

(2) The rosette of tubules (*ne. t.*) consists of from ten to fifteen loops. Each "loop" is a tubule bent upon itself in a U-shape, the apex of the U being free, and the two limbs of the loop spirally wound round each other (fig. 21). The constitution of one of these tubules is seen in figs. 31, 32, 33, Pl. XVI *bis*. Passing along the inner side of a loop is an intracellular lumen with rather a greater diameter than the others, this I call the "main lumen" (*l*). It pierces a series of granular cells, the whole of whose diameter it does not occupy, so that the wall is fairly thick and noticeably granular, the granules appearing to have a somewhat radial arrangement (fig. 33). Outside this lumen there runs, parallel with it, a "secondary lumen" (*l′*). This lumen occupies nearly the whole diameter of the cells through which it passes, so that its walls are thin and are not granular. These two are closely bound by connective tissue, amongst which run the "smaller lumina" (*l″*), forming a copious network round the other two lumina (fig. 31); like that of the secondary lumen their walls are thin and non-granular, their diameter varies, but is smaller than either of the other two lumina. The main lumina, near the

vesicle, are surrounded by a loose connective tissue whose large cells have definite boundaries (Pl. XVI *bis*, fig. 32); immediately round the "drain-pipe" cell of the lumen is some fibrous connective tissue, whose flat nuclei are shown in fig. 32; this forms a "sheath" (*ct'*) to the main lumen, though I have not found it farther away from the vesicle. Outside this sheath is the looser connective tissue. In this region only the main lumina exist. But farther away from the vesicle the "secondary" and "smaller lumina" commence (fig. 33), the whole set of lumina, forming one limb of a loop, is surrounded by a granular connective tissue whose limits are not well defined. Nuclei are scattered about, and blood-vessels are seen cut through in a section; but whether these blood-vessels actually pierce the nephridial cells, or pass between them, I am unable to say. Round the short lumen leading through the septum to the funnel is a looser connective tissue like that near the vesicle.

The course and communication of the various lumina I have not as yet followed out. Whether only one "main lumen," or whether several communicate with the vesicle, I am likewise as yet uncertain, as also whence the lumen to the funnel springs.

(3) The internal opening (*ne.f.*) (Pl. XVI, fig. 22) lies in the somite preceding that in which the tubules are placed. A narrow duct leads through the septum, and having passed this, the connective tissue round it assumes a looser form, and is lobed; the internal opening does not present itself in the form of an expanded funnel as in Lumbricus; it may be easily overlooked, as it is very small. The cilia (Pl. XVI, figs. 22, 23, 24) at the internal opening are very long, and are continued for some distance down the lumen; how far they actually extend I am unable to say, but as I could see none in the tubules of the rosette they are probably confined to the lumen leading to the funnel.

The shape of the vesicle varies somewhat in different regions of the body; that described above is one from the posterior region. In somites II and III it is very much elongated; the anterior extremity ends in the external pore, whilst posteriorly it enlarges and gives off the tubules, which are there situated

exactly at the opposite end to the pore (Pl. XVI, fig. 25). In somite XII the vesicle is shorter and wider, whilst the pore is placed nearly half way between its blind end and the rosette of tubules (fig. 26). It seems probable that the whole set of loops in Microchæta is really one continuous tubule, opening into the vesicle at one extremity, and leading to the funnel at the other: the whole tubule being bent into a number of U-shaped loops, each of which is twisted round itself. The whole nephridium, though so complicated, may be compared to that of Lumbricus, by considering the smaller lumen of the latter, bent upon the larger and more glandular portion, and then wrapped and twisted; whilst the very small muscular region of Lumbricus is enlarged into the exceedingly well-marked vesicle of Microchæta.

The Nervous System.—The cerebral ganglia (Pl. XVI *bis*, figs. 34, 35, *c. g.*), or supra-pharyngeal ganglia, lie embedded in the muscular wall of the pharynx, or rather in the radiating muscles of this organ; they lie very close together, but are not fused into a single mass as in some Earthworms. A commissure (*n. com.*) passes down on each side of the pharynx, at the junction of the buccal region with the pharynx, and the two commissures unite on the ventral surface in the fourth annulus, to form the subpharyngeal ganglion.

The following ganglia lie towards the posterior part of each somite, and, as in Lumbricus, are not very distinct (Pl. XVI *bis*, fig. 36).

In each somite nerves come off both from the ganglionic enlargements and from the ventral cord itself; three pairs, usually from the former, and one pair from the latter.

The ganglion-cells are, as in Lumbricus, more or less numerous throughout the cord, not being confined to the ganglionic swellings. The three "giant-fibres" are here present, as also a sheath, but with no muscles in the sheath; neither subneural nor latero-neural vessels are present (figs. 37, 38).

In transverse sections of the nerve-cord, the ganglion-cells (*n. g. c.*) are seen to lie apparently, each in a little capsule, as it were, of connective tissue (*ct.*), which dips into, and amongst

the fibrous portion of the cord (*n. fi.*), and clearly shows its separation into two halves. Amongst the nerve-fibres round, nearly homogeneous, nuclei are seen scattered about, which probably belong to this connective tissue.

As to the position of the cerebral ganglia, since the first sub-pharyngeal ganglion lies in the fourth annulus—i. e. somite II—then the cerebral lie in the second or third annulus—i. e. somite I. Owing to the absence of septa in this region it is impossible to say, with certainty, exactly in which annulus they lie, but since they lie in front of the first subpharyngeal, the position of which is easily determined, they are at any rate in somite I. Perrier considers it a rule that the cerebral ganglia of Earthworms lie in the third somite, and never in the first; but here at any rate is an exception. Beddard mentions that the cerebral ganglia of Typhœus lie in somite II, and probably other worms will show that his rule does not invariably hold good. The question, however, occurs, Do the annuli of Microchæta correspond to the somites of Lumbricus in this region of the body?

From the cerebral ganglion, there pass three or four nerves, forwards, on each side to the prostomium (figs. 34, 35, *np.*). From the commissure, close to the cerebral ganglia, there pass forward three or four nerves, for the number varies in the two specimens, to the wall of the buccal region (*n. B.*); from nearly the same position, but passing backwards, are nerves to the pharynx; and similar pharyngeal nerves come off from the hinder part of the commissure, both dorsally and ventrally (*n. ph*); these probably enter a "visceral system" in the alimentary canal, which I have not followed out.

Summary.—The chief points which are new or noticeable about Microchæta are as follows:

(1) The small prostomium.

(2) The numerous annuli that make up a somite, more especially in the case of the anterior somites.

(3) The small size of the setæ, relative to the size of the worm.

(4) The large size of the nephridiopores, and their arrangement in a line with the lateral setæ.

(5) The very large size, and complicated structure, of the nephridia themselves.

(6) The excessively strong septa of the anterior somites, being much thicker than those figured for other large Earthworms.

(7) The great number and small size of the spermathecæ.

(8) The position of the spermathecæ behind the other genital organs, and the presence of more than one pair in a somite.

(9) The intestinal gland in somite IX, with a structure similar to that of the calciferous œsophageal glands of Lumbricus agricola.

(10) The bifurcation of the dorsal trunk in each of the anterior somites (IV to VIII), and the union of these divisions before passing through the anterior septa of these somites.

(11) The great enlargement and thickening of the wall of the dorsal vessel in somite VIII.

(12) The curious structures, with unknown function, in somite XII.

(13) The position of the supra-pharyngeal ganglion in the somite I.

(14) The absence of a sub-neural blood-vessel.

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EXPLANATION OF PLATES XV, XVI, & XVI *bis*,

Illustrating Mr. Benham's "Studies of Earthworms."

References.

B. Buccal region. *b. m.* Basement membrane of epidermis. *b. r.* Branch of the dorsal trunk which passes down parallel with the circumpharyngeal nerve commissure. *b. v.* Blood-vessels. *b. w.* Body wall. *c.* Drain-pipe cell forming the wall of the nephridial lumen. *cap.* Capillaries. *c. g.* Cerebral

ganglion. *ci.* Cilia. *cœ.* Cœlom. *cœ. ep. pa.* Somatic portion of cœlomic epithelium. *c. e. vis.* Splanchnic portion of cœlomic epithelium. *clb.* Club-shaped cells of Clitellum. *col.* Columnar cells of epidermis. *com.* Commissural vessels. *com'.* Lateral hearts. *com. al.* Branches from commissural vessel of somite IV to œsophagus. *com. spt.* Septal branches of commissural vessels. *cr.* Ciliated rosette. *ct.* Connective tissue. *cu.* Cuticle. *c. v.* Blind end of nephridial vesicle. *D.* Dorsal trunk of the vascular system. *d d.* Portions where the dorsal trunk has divided into two parallel vessels. *d. gl.* Duct of multo-cellular gland. *d. int.* Intestinal branches from the dorsal trunk. *d. spt.* Septal branches from dorsal trunk. *d. sw.* Heart-shaped double-swelling on dorsal trunk in somite VIII. *e. n.* Narrow, elongated cells of Clitellum. *G.* Gizzard. *g.* Subdivision of gizzard. *gang.* Ganglia on ventral nerve-chain. *gb.* Goblet cells in the epidermis. *g. f.* Giant-fibres. *gl. c.* Glandular cells in the intestinal epithelium. *gl. int.* Gland on the intestine in somite IX. *int. ep.* Epithelium of intestine. *int. s.* Sacculated intestine. *int. t.* Tubular intestine. *i. w.* Wall of intestine. *L.* Lateral trunk of vascular system. *l.* Main lumen of nephridial tubule. *l'.* Secondary lumen. *l''.* Smaller lumina. *l. al.* Branches from lateral trunk to alimentary tract. *l. spt.* Septal branches from lateral trunk. *l. w.* Longitudinal vessel in the body wall. *m.* Muscle. *mlr. gl.* Multicellular gland. *mus. cir.* Circular muscles. *mus. lg.* Longitudinal muscles. *n.* Nucleus. *n. B.* Nerves to buccal region. *n. c.* Ventral nerve-chain. *n. com.* Circumpharyngeal nerve commissure. *ne. f.* Nephridial funnel. *ne. m.* Sphincter muscle at nephridiopore. *ne. o.* Nephridiopore. *neph.* Nephridium. *ne. t.* Tubules of nephridium. *ne. v.* Vesicle of nephridium. *n. fi.* Nerve-fibres. *n. g.* Ganglion. *n. gc.* Nerve ganglion-cell. *n. l.* Lateral nerves. *n. p.* Nerves to prostomium. *n. ph.* Nerves to pharynx. *nw. gl.* Vascular network on intestinal gland. *nw. ph.* Vascular network on pharynx. *O.* Ovary. *Oe.* Œsophagus. *œ. p.* Brown pigment round œsophagus. *ov.* Ovum. *ov. l.* Lobules of the ovary. *p.* Pores through cuticle of goblet cells. *Ph.* Pharynx. *ph. v.*, *ph. v'.* Branches of the dorsal trunk on the pharynx. *rm.* Radiating muscles of the pharynx. *r. s.* Rosette-sac. *s.* Septum. *sem. res.* Seminal reservoir. *s. l.* Lateral setæ. *sp. a.* Spermatozoa. *spd.* Sperm duct. *spo.* Sperm pore. *spt.* Branch to septum from pharyngeal vessel. *spth.* Spermathecæ. *spth. p.* Aperture of spermathecæ. *s. v.* Ventral setæ. *T.* Typhlosolian trunk of vascular system. *t.* Testis. *t. int.* Intestinal branches from typhlosolian trunk. *t. pr.* Anterior prolongation of seminal reservoir which encloses the testis. *ty.* Typhlosole. *V.* Ventral trunk of vascular system. *va.* Valve at the entrance of septal branch into dorsal trunk. *va. c.* Valve at exit of commissural vessel. *va. i.* Valve at exit of intestinal vessel. *v. int.* Intestinal branch of ventral trunk. *v. spt.* Septal branch of ventral trunk. *v. t.*, *v. t'.* Branches from ventral trunk to seminal reservoirs. *w. sp.* Wall of spermatheca. *x.* Funnel-shaped organ of unknown function. *xp.* Ciliated processes of funnel-shaped organ. *y.* Glandular organ of unknown function.

FIG. 1.—External view of the body wall, cut along the dorsal mid-line and pinned out. (Natural size.) I to XXVIII: the first twenty-eight somites, showing the variation in the number of annuli to the different somites; the ventral edges and the folds in the sides of the Clitellum are shown. *m.* Mouth. *m. p.* Male pore. *ne. o.* Nephridiopore. *pro.* Prostomium. *sl.* Lateral setæ. *sv.* Ventral setæ. *spth. p.* Pores of the spermathecæ.

FIG. 1 *a*.—A flake of the cuticle, to show the two series of striæ and the pores of the goblet-cells (*p*).

FIG. 1 *b*.—Diagrammatic outline of a transverse section through the body, to show the position and arrangement of the setæ, the relative size of which is exaggerated.

FIG. 2.—General view of the contents of the body cavity, when the body wall has been cut along the dorsal mid-line and pinned aside. (Natural size.) *C. g.* Cerebral ganglia. *Com.* Commissural blood-vessels. *Com'.* Contractile "hearts." *D.* Dorsal blood-trunk. *dd.* Doubled portion of the dorsal trunk. *dsw.* Heart-shaped dilatation of the dorsal trunk. *G.* Gizzard. *g.* Posterior portion of gizzard. *gl. int.* Intestinal glands. *int. s.* Sacculated intestine. *int. t.* Tubular intestine. *L.* Lateral longitudinal blood-trunk. *m. b.* Muscular bands passing from the septa to the body wall. *neph.* Nephridium. *ne. t.* Tubules of nephridium. *ne. v.* Vesicle of nephridium. *o.* Ovary. *œs.* Œsophagus. *ph.* Pharynx. *r. m.* Radiating muscles of the pharynx. *S.* Septum. *sem. res.* Seminal reservoirs. *spth.* Spermathecæ. *x.* Problematic organ attached to septum.

FIG. 3.—View of the alimentary tract after removal of other structures that hide it, but in its natural position with reference to the septa. (Natural size. Somewhat diagrammatic.) *B.* Buccal region. *G.* Gizzard. *g.* Posterior portion of gizzard. *gl. int.* Intestinal gland ("glande de Morren"). *int. s.* Sacculated intestine. *int. t.* Tubular intestine. *œ.* Œsophagus. *œ. p.* Pigment on œsophageal wall. *ph.* Pharynx. *r. m.* Radiating muscles of the pharynx. *S.* Septa. *ty.* Typhlosole.

FIG. 4.—View of somites VIII to XIV after the alimentary tract has been removed, so as to show the genital organs. In the posterior somites the ventral blood-trunk has been left in, whilst anteriorly it has been removed to show the nerve-cord. The nephridia have been removed, except those in somite VIII. On the right side, the seminal reservoirs have in great part been removed, to show the ciliated rosettes, and their communication with the sperm-ducts. (× 2.) *c. r.* Ciliated rosette. *n. c.* Ventral nerve-cord. *neo.* Nephridiopores. *neph.* Nephridium. *o.* Ovary. *r. s.* Rosette sac. *S.* Septum. *sem. res.* Seminal reservoir. *sp. d.* Sperm-duct. *spo.* Sperm pore. *spth.* Spermathecæ. (The lithographer has drawn them rather too large.) *tpr.* Testicular process of the seminal reservoir. *V.* The ventral blood-trunk. *V. spt.* Septal branch of the ventral trunk. *x. y.* Problematic organs in somite XII.

FIG. 5.—The dorsal trunk and longitudinal lateral trunks, with their main

branches. *br.* One of the two branches into which the dorsal trunk divides just behind the cerebral ganglion, and which passes round the pharynx into the ventral trunk. *c.g.* Cerebral ganglia. *com.* Commissural vessel from the dorsal to the ventral trunk. *com'.* Similar vessel, but here ampullated and called "lateral heart." *com. al.* Vessel from commissural vessel to the œsophagus. *com. spt.* Vessel from commissural vessel to the septum and body wall. *D.* The dorsal trunk. *dd.* Doubled portion of the dorsal trunk. *d. int.* Vessel from the dorsal trunk to the intestine. *d. spt.* Vessel from the septum and body wall to the dorsal trunk. *dsw.* Heart-shaped swelling on the dorsal trunk. *L.* Longitudinal lateral trunk. *l. œ.* Vessel from the longitudinal lateral trunk to the œsophagus. *l. spt.* Vessel from the longitudinal lateral trunk to the septum and body wall. *lw.* Longitudinal vessel in the body wall: this is rather diagrammatic, and represents the network as well as other longitudinal vessels here. *nw. gl.* Branches which go to form a network on the wall of the intestinal gland. *nw. ph.* Branches from the dorsal, ventral, and longitudinal lateral trunks which go to form a network on the wall of the pharynx. *ph. v.* Vessel in somite III, from the dorsal trunk to the pharynx. *ph. v.* Similar vessel of somite II (these two go to form the network on the pharynx). *spt.* Vessel from *ph. v.* to the first septum.

Fig 6.—Side view of the vascular system, the side of the body wall being supposed to have been removed. *br.* One of the branches, into which the dorsal trunk divides just behind the cerebral ganglion, which passes down alongside the cerebral commissure to the ventral trunk. *b. w.* Cut edge of the body wall. *c. g.* Cerebral ganglion. *com.* Commissural vessel. *com'.* "Lateral hearts." *com. al.* Vessel from the commissural vessel to the œsophagus. *com. spt.* Vessel from the commissural vessel to the septum. *D.* Dorsal trunk. *d. int.* Vessel from the dorsal trunk to the wall of the intestine. *d. spt.* Vessel from septum and body wall to the dorsal trunk. *L.* Longitudinal lateral trunk. *l. al.* Vessel from the longitudinal lateral trunk to the alimentary canal. *l. spt.* Vessel from the longitudinal lateral trunk to the septum, &c. *l. w.* Vessels in the body wall. *n. c.* Ventral nerve-cord. *n. com.* Nerve commissure from the cerebral to the infra-pharyngeal ganglion. *nw. gl.* Vessels from the longitudinal lateral trunk going to form a network on the intestinal gland. *nw. ph.* Vessels from the longitudinal trunks going to form a network on the pharynx. *ph. v.* Vessel from the dorsal trunk, helping to form the network on the pharynx. *ph. v.'* Similar vessel in somite II. *S.* Septum. *spt.* Branch from *ph. v.* to the first septum. *T.* Typhlosolar trunk. *t. int.* Vessel from the typhlosolar trunk to the intestinal wall. *V.* Ventral trunk. *v. int.* Vessels from the ventral trunk to intestinal wall. *v. spt.* Vessels from the ventral trunk to the septum and body wall. *v. t.*, *v. t'.* Vessels from the ventral trunk to the seminal reservoirs.

Fig. 7.—Problematic funnel-shaped organ attached to the anterior face of the posterior septum (*s.*) of somite XII, and labelled (*x*) in Fig. 4. (× 12.)

Fig. 8.—Problematic organ attached to the posterior face of the anterior septum (*s.*) of somite XII, labelled (*y*) in Fig. 4. (× 10.)

Fig. 9.—A spermatheca, seen as a transparent body when mounted in glycerine. *spa.* The spermatozoa within it. *sptho.* The fixed end of the organ, leading to the exterior. *w. sp.* The muscular wall, lined within by columnar epithelium.

Fig. 10.—A spermatozoon.

Fig. 11.—A portion of a section through a seminal reservoir, showing the thick trabeculæ (*t. ct.*), which divides the cavity (*cav.*) up into small chambers. *b. v.* Blood-vessels traversing the trabeculæ. *spc.* The developing spermatozoa.

Fig. 12.—A transverse section through the testicular prolongation of the seminal reservoir, showing the enclosed testis (*t.*) attached along its outer wall. *sem. res.* A portion of the adjoining seminal reservoir.

Fig. 13.—A more enlarged and detailed drawing of the same. (The lithographer should have made this relatively larger than Fig. 12.) *bv.* Blood-vessel from the wall of the sac passing into the substance of the testis. *cav.* cavity of the sac. *c. t.* Connective tissue of the wall of the sac. *spm.* Sperm-mother-cells.

Fig. 14.—A portion of the septum (*s.*) between somites XII and XIII, together with the ovary, on its posterior face, and the problematic organ (*x*, in Fig. 4) on its anterior face. Each lobule of the ovary (*ov. l.*) is made up of cœlomic epithelial cells (*c. ep.*), which are not shown, and ova (*ov.*). The edge of the funnel-shaped organ (Fig. 7) is fringed with ciliated processes (*xp*), of which a few are here shown.

Fig. 15.—The heart-shaped swelling of the dorsal trunk in somite VIII, seen from the dorsal surface. *g.* An apparent groove, which is really only due to the close approximation of the two parts of the double dorsal trunk. *S. S.* The septa.

Fig. 16.—The heart-shaped organ after removal of its dorsal walls, to show its double character and thickened walls. The two halves have been stretched apart slightly, in order to show that the groove is really a division. *com'.* A portion of a lateral heart, leaving the dorsal vessel just behind a valve (*va. c.*), in each side. Similar valves (*va. c.*) are shown in the anterior part of the dorsal vessel. *S.* Septum.

Fig. 17.—A portion of the dorsal trunk (*D.*) laid open, to show the valves within. *d. spt.* A vessel from the septum (*m*) entering the dorsal trunk anteriorly to the small valve (*va.*). The vessel to the intestine (*d. int.*) leaves the trunk just behind the valve (*va. i.*).

Fig. 18.—A portion of a "lateral heart." *m.* A band of circular muscles causing the ampullate appearance. *pr.* The narrow proximal portion where it is joined to the dorsal trunk.

Fig. 19.—A diagrammatic section through the body, to show the arrangement of the vascular trunks anteriorly to somite XI. The arrows indicate the

course of the blood. *al.* Alimentary canal. *b. w.* Body wall. *cœ.* Cœlom. *Com.* "Lateral heart." *com. spt.* Vessel to body wall from the lateral heart. *D.* Dorsal trunk. *i. w.* Wall of the alimentary canal. *L.* Longitudinal lateral trunk. *L. al.* Vessel from the alimentary canal to the lateral trunk. *L. spt.* Vessel from septum and body wall to lateral trunk. *n. c.* Nerve-cord. *V.* Ventral trunk. *V. spt.* Vessels from the ventral trunk to septum and body wall.

Fig. 20.—A diagrammatic section through the body, to show the arrangement of the vascular trunks posteriorly to somite xiii. The arrows indicate the course of the blood. *al.* Alimentary canal. *b. w.* Body wall. *cœ.* Cœlom. *D.* Dorsal trunk. *d. int.* Vessel from the dorsal trunk to the wall of intestine. *d. spt.* Vessel from septum, &c., to the dorsal trunk. *i. w.* Wall of intestine, with network of vessels on it. *n. c.* Nerve-cord. *T.* Typhlosolar trunk. *t. int.* Vessel from wall of intestine to typhlosolar trunk. *ty.* Typhlosole. *V.* Ventral trunk. *V. int.* Vessel from ventral trunk to enter the network on the wall of intestine. *V. spt.* Vessel from ventral trunk to body wall, &c. The vertical vessel from typhlosole to dorsal trunk is not lettered.

Fig. 21.—A complete nephridium. *c. v.* Blind end of nephridial vesicle. *ne. f.* Nephridial funnel. *ne. m.* Sphincter muscle of the nephridiopore (*ne. o.*). *ne. t.* Nephridial tubules, much twisted and forming a rosette-shaped mass. *ne. v.* Nephridial vesicle.

Fig. 22.—The nephridial funnel and neighbouring portion of a tubule after passing through the septum. *ci.* Long cilia at the internal opening of the funnel. *c. t.* Connective tissue round the lumen (*l.*) of the tubule.

Fig. 23.—A more enlarged drawing of the funnel, showing large connective-tissue cells. Letters as before. (Drawn by A. G. Bourne.)

Fig. 24.—Four ciliated columnar cells from the nephridial funnel. *ci.* Cilia. *n.* Nuclei.

Fig. 25.—The first nephridium, showing the much elongated region of the vesicle between the tubules and the nephridiopore. *c. v.* Blind end of vesicle (*ne. v.*). *ne. t.* Bunch of tubules. *ne. o.* Nephridiopore.

Fig. 26.—A nephridium from somite xii, showing the position of the nephridiopore (*ne. o.*) about half way between the tubules (*ne. t.*) and the blind end (*c. v.*).

Fig. 27.—Interior of the wall of the œsophagus, showing the ridges and papillæ.

Fig. 28.—Elongated seta, from the anterior region of the body.

Fig. 29.—Seta from the ventral series in the posterior region of the body.

Fig. 30.—Has been erased.

Fig. 31.—The free end of a loop of a nephridial tubule, viewed as a transparent object, when mounted in glycerine. *c. t.* The connective tissue surrounding the lumina and binding them together. *l.* The main lumen with thickish walls, and lying parallel to (*l'*) the secondary lumen. *l''.* The smaller lumina, forming a network round the other lumina, surrounded by connective tissue.

Fig. 32.—A transverse section across a nephridial tubule near its origin from the vesicle. Three main lumina (*l.*) are shown. There are here no smaller lumina, and the connective tissue (*c. t.*) is somewhat vesicular, and the cell boundaries are evident. *bv.* are the blood-vessels in the connective tissue. Round the pierced cells of the tubule is a sort of sheath of flat cells (*ct'.*) whose nuclei are seen.

Fig. 33.—A section across a nephridial tubule further away from the vesicle. A main lumen (*l.*) is seen with a thick wall, corresponding to *l.* in Fig. 32; and in addition are secondary (l'.) and smaller lumina (l''.) which communicate with one another. The boundaries of the connective-tissue cells are not evident in this region, but their nuclei (*n.*) are shown. *bv.* are the blood-vessels.

Fig. 34.—The cerebral and two ventral nerve ganglia from above, showing the nerves coming from them. *c. g.* Cerebral ganglia. *n. B.* Nerves from the commissure (*n. com.*) to the buccal region. *n.* g'., *n.* g''. The first and second ganglia of the ventral chain. *n. l.* Lateral nerves from the ganglia. *n. p.* Nerves from the cerebral ganglia to the prostomium. *n. ph.* Nerves from the cerebral ganglia and from the commissure to the pharynx.

Fig. 35.—The cerebral and first ventral ganglia from the side, together with the buccal region (*B.*) and pharynx (*ph.*). Letters as before.

Fig. 36.—A portion of the ventral nerve-cord, with three ganglia (*gang.*), and the lateral nerves (*n. l.*) coming off, some from the ganglia, others from the nerve-cord.

Fig. 37.—Transverse section through a ganglion. *c. ep.* Nuclei of the cœlomic epithelium. *c. t.* Connective tissue. *g. f.* Three giant-fibres. *m.* Sections of longitudinal muscles at each side. *n. g. c.* Ganglion-cells, each lying apparently in a separate space in connective tissue. *n. fi.* Nerve-fibres. *n. l.* Lateral nerve leaving the ganglion. *sh.* Sheath of the nerve-cord, without muscles.

Fig. 38.—Transverse section through the nerve-cord between two ganglia. Letters as before. *n.* Nuclei of connective tissue.

Fig. 39.—A portion of a transverse section through the body wall. *bv.* Blood-vessels. *col.* Columnar epithelial cells. *cœ. ep. par.* parietal cœlomic epithelium. *c. t.* Connective tissue between the muscle strands, and forming spaces in which the longitudinal fibres lie. *cu.* Cuticle. *d. gl.* Ducts of the deep-lying multicellular glands; these ducts pass into, and probably through, the epidermic layer. *gb.* Goblet-cells of the epidermis. *lg. f.* Cut ends of fibres of the longitudinal muscles. *mlr. g.* Multicellular glands, lying in spaces amongst the longitudinal muscles. *mus. circ.* Circular muscular layer *mus. lg.* Longitudinal muscular layer. *m. b.* Basement membrane. *P. b.* Goblet-cell.

Fig. 40.—A portion of the epidermis much more enlarged, showing the contents of the goblet-cells and the network of their protoplasm (*b. c.*). In some cases there appear to be more than one nucleus in these cells (as at *a.*),

but often there is the appearance of a very delicate membrane separating one part from another of these cells. *b.* is a cell which has emptied its granular contents. *b. m.* Basement membrane. *col.* Columnar cells. *cu.* Cuticle. *gb.* Goblet-cell. *n.* Nucleus. *nn.* Nuclei of cells in a different layer. *p.* Pore of goblet-cell.

FIG. 41.—A portion of a section through the modified epidermis of the clitellum, showing the two other varieties of cells beside the columnar (*col.*) and the goblet-cells (*gb.*). *clb.* The club-shaped cells, filled with very minute granules, which are not very well represented. *e. n.* Narrow elongated cells with similar contents to the ordinary goblet-cells. *cap.* Blood-vessel passing up between the columns of cells. *c. t.* Vertical strands of connective tissue separating the club-shaped cells into columns, and spreading out below them; and also below the ordinary epidermic cells to form a network, in which the blood-vessels of this region lie.

FIG. 42.—A portion of a section through the wall of the intestine. *bv.* The vascular network cut across. C. E. *vis.* Visceral cœlomic epithelium, with numerous yellow granules in the cells. *c. t.* Connective tissue round the muscles. *gl. c.* Gland-cells of the intestinal epithelium. *int. ep.* Ciliated columnar cells of the epithelium. *mus. circ.* Circular muscular layer. *mus. lg.* Longitudinal muscular layer. *n.* Nuclei. *w.* Wall of blood-vessels.

The Official Refutation of Dr. Robert Koch's Theory of Cholera and Commas.

The following Memorandum has been drawn up by a Committee convened by the Secretary of State for India, for the purpose of taking into consideration a Report by Drs. E. Klein and Heneage Gibbes, entitled "An Inquiry into the Etiology of Asiatic Cholera." The members of this Committee were—Dr. William Aitken, F.R.S., Professor of Pathology, Army Medical School; Dr. J. Burdon-Sanderson, LL.D., F.R.S., Waynflete Professor of Physiology, Oxford; Dr. Norman Chevers, C.I.E., Deputy-Surgeon General, late Principal and Professor of Medicine, Medical College, Calcutta; Dr. F. de Chaumont, F.R.S., Professor of Hygiene, Army Medical School; Sir Joseph Fayrer, K.C.S.I., LL.D., F.R.S., Surgeon-General, Honorary Physician to Her Majesty the Queen and to His Royal Highness the Prince of Wales, Physician to the Council of India; Sir William W. Gull, D.C.L., LL.D., F.R.S., Physician Extraordinary to Her Majesty the Queen, Physician in Ordinary to His Royal Highness the Prince of Wales; Sir W. Guyer Hunter, K.C.M.G., Surgeon-General, Honorary Surgeon to Her Majesty the Queen, late Principal and Professor of Medicine, Medical College, Bombay; Sir William Jenner (President), K.C.B., D.C.L., LL.D., F.R.S., Physician in Ordinary to Her Majesty the Queen and to His Royal Highness the Prince of Wales, President of the Royal College of Physicians; Dr. Timothy Richards Lewis, Surgeon-Major, Assistant-Professor of Pathology, Army Medical School; Dr. John Macpherson, Inspector-General of Hospitals (retired); Dr. Jeffery A. Marston, Deputy Surgeon-General, Head of Sanitary Branch, Army Medical Department; Sir William R. E. Smart, K.C.B.,

K.L.H., Inspector General of Hospitals and Fleets (retired), Honorary Physician to Her Majesty the Queen; Dr. John Sutherland, Member of the Army Sanitary Commission. Dr. Timothy Lewis, acted as Secretary:

1. The epidemic outbreak of cholera which occurred in Egypt about two years ago gave a fresh impetus to the study of the etiology and pathology of the disease, and special measures were taken by the Governments of Germany and France, as well as by our own, to elucidate the matter during the continuance of the epidemic. The labours of the German Commission (of which Dr. Robert Koch was the chief) attracted exceptional attention, from the circumstance that it was believed that a specific organism—a bacillus, resembling one which had been found in glanders—had been discovered by them, which warranted the assumption that further study would be likely to demonstrate that it was the special cause of the disease.

2. With this object in view, the German Commission proceeded to India towards the latter end of 1883, and early in 1884 Dr. Robert Koch announced that this organism—now described, however, as curved, or comma-shaped, and not straight—must in reality be looked upon as the essential cause of cholera, on the grounds, principally, that it was always present in the alvine discharges in this disease, and in the mucous tissue of the lower part of the small intestine; that it was not to be found under any other conditions; and that a causal connexion between the organism and the disease had been demonstrated by the circumstances that comma-shaped organisms had been found in a tank in Calcutta near a village in which the people suffered from cholera, and that the disease diminished simultaneously with the diminution of the commas from the water of the tank.

3. As it was obvious that, in view of the new light which was very generally supposed to have been shed on the etiology of cholera, other prophylactic and curative measures would have to be adopted in the event of the statements being confirmed, and that harm might result were such measures resorted to on erroneous grounds, Sir Joseph Fayrer (in his

capacity as Physician to the Council of India) suggested to the Secretary of State in May, 1884, that the Government should institute a special inquiry into the whole subject. This proposal was acceded to, and it was arranged that two gentlemen, who were exceptionally well qualified to conduct researches of this character, Drs. Klein and Gibbes, should proceed to India at their earliest convenience. Every possible assistance was to be accorded to them, both at home and in India, for the prosecution of their investigations, and they were instructed to furnish a report to the Government on the conclusion of their labours, which was afterwards to be submitted to the consideration and final judgment of a Committee appointed by the Secretary of State for India in Council.

4. Drs. Klein and Gibbes embarked for India on August 6, 1884, and visited Bombay, Calcutta, and other cities in that country for the purpose of studying the disease. They left again for England on December 12, 1884, and towards the end of March, 1885, submitted an account of their researches to the Secretary of State for India.

5. Such, briefly stated, appear to be the main incidents which have given rise to the preparation of the report, which has been submitted to the consideration of this Committee, copies of which were placed in the hands of the members under cover of India Office letter of June 17, 1885. The 'Proceedings' will be found to contain a brief summary of the remarks made by individual members at the meetings, together with some notes bearing on the subject under discussion which were handed to the secretary, and are reproduced in the form of an appendix.

6. While fully accepting the truth of the statement that choleraic dejections are generally characterised by the presence of comma-shaped organisms, as maintained by Dr. Koch, a perusal of the report shows that Drs. Klein and Gibbes directly traverse several of Dr. Koch's conclusions, and, in some cases, his statements as to assumed matters of fact. Indeed, the correctness of what may be conveniently described as Dr. Koch's three main propositions is emphatically denied by Drs.

Klein and Gibbes. Stated shortly, Dr. Koch appears to maintain—first, that the number of comma-shaped organisms in the intestinal tissues and contents is in proportion to the acuteness of the attack, and that these organisms generate within the body a ferment by which the system is poisoned: second, that they are not found under any conditions other than in connection with cholera; and, third, that their presence in a tank which supplied certain cholera-affected villages in Calcutta with water was, practically, a proof of the causal connection between the organisms and the disease.

7. With regard to the first cited of these propositions, Drs. Klein and Gibbes write as follows:

"Comma-bacilli are present in the rice-water stools of cholera patients, but their number is subject to very great variations; while in some they are easily found, in others it is difficult to meet with one" (p. 6). . . "In order to explain the causation of the disease by the comma-bacillus, Koch assumes that, it being absent from the blood and present only in the small intestine, a chemical ferment, which is the actual poison, is secreted by it, and on the amount of this the severity and rapidity of the illness depend; in the typical acute cases a large amount of this chemical ferment is being produced, absorbed by the system, and therefore death rapidly ensues. And this, Koch states, is in accordance with the observation made by him that in these instances the comma-bacilli are so numerously found in the mucous membrane itself, particularly in the lower part of the ileum, that this appears almost like a pure cultivation of the bacilli. If this were really the case—viz. if it could be shown that in acute typical cases of cholera not only the flakes composed of the detached epithelium and mucus, found in the cavity of the intestine and on the surface of the mucous membrane, but also, as Koch states, the superficial layers of the mucous membrane of the congested ileum, are loaded with comma-bacilli and nothing else, this would be a remarkable fact, and there would be strong grounds for believing that the comma-bacilli must in some way or another be related to the morbid process, although it would not neces-

sarily follow that these bacilli must, as a conditio sine quâ non, be the actual cause of the disorder.

"Now, our observations are in direct opposition to these statements of Koch. It is difficult to explain how such a statement could have been made. Several cases of acute typical cholera were subjects of post-mortem examination. Death had followed in some within from sixteen to twenty-eight, in others from eight to twelve hours; the post-mortem was made in some within one, in others within half or a quarter of an hour. The ileum, and, as a matter of fact, the whole of the small intestine, was either slightly and uniformly injected and its mucous membrane slightly tumefied, the cavity both of the jejunum and ileum being filled with clear watery fluid in which were suspended large numbers of the typical flakes; there was no difference noticeable in this respect between the lower part of the ileum and the rest of the small intestine. In a few cases in the lower portion of the ileum the solitary follicles and Peyer's glands were distinct, and presented either a slight redness or only redness at the margin. Koch's statement that in acute typical cases of cholera the Peyer's glands and solitary glands of the ileum are enlarged, and on naked-eye inspection already visible by a slight injection of their marginal portion, is not confirmed by our observations, since several acute typical cases came under our observation in which such a condition was not noticeable, that is to say, cases coming under the category of the pure typical cases of Koch in which the mucous membrane ought to be almost 'a pure culture of comma-bacilli'" (pp. 7, 8).

"That the comma-bacilli should in some cases of cholera, particularly those with typical rice-water stools, with or without many mucous flakes, be very abundant may simply mean that here the comma-bacillus finds the most suitable conditions for growth, more suitable than any other bacillus, although, as a matter of fact, we have not found that, except in a few cases, it always predominates over other bacilli, particularly very short, thin, straight bacilli, to be mentioned below. The statement of Koch that, in acute typical cases, the comma-

bacilli are found chiefly and almost exclusively in the mucus flakes of the lower part of the ileum—a statement borne out by our observations—does not harmonise, it appears, with the assumption that the comma-bacilli are the cause of the disease, since, in several acute typical cases, there is no difference as regards the aspect of the intestine, the amount of fluid and flakes contained in the cavity of the intestine, and the anatomical changes of the membrane between the lower and upper portions of the ileum as well as jejunum" (p. 7). . . . .

"Fine sections made of the mucous membrane of the above typical acute cases of cholera, after hardening the intestines in alcohol or Müller's fluid, particularly the first (also used by Koch), and stained in various aniline dyes (gentian violet, in several modifications, Spiller's purple, methyl blue, magenta, after Ehrlich's, Weigert's, Koch's, and other methods), revealed the total absence of comma-bacilli from the mucous membrane itself, from the tissue of the villi, from the Lieberkühn's follicles, and from the lymphatic tissue of the Peyer's and solitary glands; the epithelium of the surface of the villi having become detached, during life has not generally kept its place in the hardened intestine but in many places the epithelium of the surface as well as that of the Lieberkühn's follicles, although loosened and slightly raised from the mucous membrane, had nevertheless kept its position and was fixed during the hardening; and in these places the comma-bacilli or any other organisms are conspicuous by their absence; they are nowhere to be found, they are simply absent" (page 9). . . .

"Some of the ardent supporters of Koch's theory, after it has been shown that the mucous membrane of the ileum or of any other part in the acute cases of cholera, provided the examination be made immediately or very soon after death, is absolutely free of comma-bacilli, might and probably will, nevertheless, cling to the comma-bacilli as the cause of cholera, saying,—'But the comma-bacilli are present in the cavity of the intestine, and although absent from the mucosa itself might nevertheless be the producers of the chemical ferment, seeing that they are present in such large numbers.' As answer to

this it may be repeated :—(1) That there are acute cases in which the comma-bacilli are very scarce indeed, even after the disease has well set in; that they should have been present in sufficiently large numbers in the lower part of the ileum before the symptoms appeared, in order to produce the large amount of chemical ferment which is to be absorbed—for this is what is meant by absorption of the chemical ferment, for no absorption can go on in an intestine during the attack itself, when the wall of the stomach and intestines discharge such enormous quantities of fluid as fast as they can—must be evident to every one to be an absurdity; an assumption of this kind would imply that the comma-bacilli are present in the fæcal matter in the lower part of the ileum before the setting in of the disease, and consequently they would have to remain here long enough to produce the virus, but for such an assumption there is not a tittle of evidence, and all our knowledge of the physiology of the intestine is against it; (2) that the whole of the small intestine presents in some acute typical cases the same appearances—viz. slight congestion, the cavity filled with clear fluid, in which are suspended the typical mucus flakes, and the great scarcity indeed of comma-bacilli in the flakes taken from the jejunum and upper part of the ileum; and (3) that the comma-baccilli are present only in dead tissues—for the mucus flakes are in all respects dead tissue, and they are found more numerously the lower down we go in the cavity of the ileum; these two facts point clearly to the comma-bacilli being putrefactive organisms" (page 11). . . .

"The blood of cholera patients has been carefully examined in the fresh state, on stained specimens, and by cultivation; the blood was obtained according to the usual approved method from patients in various stages of the disease, from ten hours after seizure to forty-eight hours, and in no one single instance could the presence of any kind of bacterium or other organism be shown to exist in the blood. The preparations examined fresh, those examined after staining with aniline dyes, revealed nothing that could be identified either as extraneous matter, or as in any way indicating a specific morphological

change; all assertions to the contrary must be put down as based on imperfect method of examination or insufficient acquaintance with the appearances of blood in health and disease" (page 16). . . .

"Numerous cultivations were made with the juice of the mesenteric glands, but no trace of bacteria was obtained, except in those tubes in which clearly and unmistakeably putrefactive micrococci or putrefactive thickish bacilli had found their entrance. Thus, then, as regards the blood and tissues, the conclusion is imperative that no kind of bacteria are present in patients suffering from cholera" (page 17). . . .

8. The foregoing extracts, especially when taken in connection with observations recorded by other observers, appear to justify the inference that no direct relation exists between the number of comma-shaped organisms associated with the choleraic process and the gravity of the disease, and that these organisms are not found in the blood or tissues, and are not ordinarily, if ever, to be found in the tissues of any part of the intestinal canal in even the most acute cases of cholera when the post-mortem examination is made immediately after death.

9. Passing on to the second of the above formulated propositions—that comma-bacilli are not found under any conditions other than cholera—Drs. Klein and Gibbes assert that "this cholera bacillus, or at any rate one that in morphological respects appears identical with it, occurs also in the stools of cases of diarrhœa. In an epidemic of diarrhœa that occurred in the autumn of 1883 in Cornwall, the stools of the patients contained . . . curved organisms which it is impossible to distinguish from the comma-bacillus of cholera stools; in size they are the same, in being curved they are the same, and, just as is the case with the choleraic comma-bacilli, some examples are either slightly pointed at the ends or blunt. They occurred not less numerously than they are sometimes found in cholera stools" (page 7). They were also met with in cases of dysentery and enteric catarrh, and "in a case of chronic phthisis of which a post-mortem examination was made, the mucus of the small intestine, although

free of any tubercle bacilli, contained, besides other putrefactive organisms, also comma-bacilli, and in this case they were so distinct that there was no difficulty in identifying them, and they were as numerous as in many cholera stools that we have examined. In the stool of a case of diarrhœa of a child suffering from chronic peritonitis (February, 1882) there are present in specimens stained with Spiller's purple numbers of comma-bacilli which it is impossible to distinguish from choleraic comma-bacilli; in size, shape, and general aspect they appear identical. On the whole, then, we maintain, contrary to Koch's emphatic statement, that the comma-bacilli occur also in cases of intestinal disease other than cholera" (page 7).

10. Both before and since this report was written evidence of a like character has been adduced, but it is to be borne in mind that, in at least some instances, such, for example, as the comma-shaped organisms which have been found associated with the cases of so-called "cholera nostras" in Bonn, it has been stated that "although in size and form they resemble those of cholera, they are, neverthelsss, not identical with them." Drs. Klein and Gibbes draw attention to the fact, which had been recently pointed out, that comma-shaped bacilli, similar in appearance to those found in cholera, are ordinarily present in certain parts of the alimentary tract in health; and, as will be seen by a reference to our 'Proceedings,' there is reason to assume that these comma-shaped organisms present themselves under two, if not three, forms in the mouth alone. It has latterly been shown by Miller that at least one of these forms can be cultivated, though isolating it in the first instance appears to have been a difficult task; and we are informed that Dr. Klein (apparently since the submission of the report) has succeeded in cultivating either this or one of the other forms of mouth-commas, and has, moreover, demonstrated that its action on the media in which it grows is identical with that of the comma-bacillus as derived directly from a case of cholera. That the two forms are absolutely identical does not, however, appear to have been definitely

established. Still the facts which have been brought to the notice of the Committee seem to point to the probability that the special organism to which such virulent properties are ascribed by Dr. Koch will, sooner or later, be demonstrated to be one or other of the various curved forms ordinarily found in the alimentary tract in health, the growth of which has been favoured by the exceptional conditions which exist in the intestine during an attack of cholera.

11. As regards the third point—the evidence as to the causal connection between the comma-shaped organisms and cholera—perhaps the most striking circumstance which gave support to the theory advanced by the German Commission in India was that referred to by Dr. Koch, that, when visiting one of the native quarters in the suburbs of Calcutta, in which an outbreak of the disease had occurred, he discovered comma-shaped bacilli in the village tank, and, further, that the disease diminished simultaneously with the diminution of the commas in the water.

12. Regarding this phase of the question, Drs. Klein and Gibbes write:

"We have had the opportunity, in connection with Dr. D. D. Cunningham, to make an examination of the water of some of the tanks in Calcutta, with reference to this very question of the comma-bacilli.

"The same tank that plays such a conspicuous part in Koch's report above mentioned was visited on the 26th November. It is surrounded by native huts in which about 200 families are living. There had occurred one case of cholera in this bustee about the first week of the month of November. The water of this tank was very dirty, particularly all along the shore, and the people around the tank, as is customary, made use of the water for all and every kind of domestic and other purposes, including drinking.

"A sample of this water was taken from near the shore, where it appeared particularly impure, about twenty yards from the house in which the cholera case had occurred, and the microscopic examination revealed undoubted comma-

bacilli, identical in every respect with those found in choleraic dejecta. Notwithstanding their presence in this water, and notwithstanding the extensive use the 200 families were constantly making of it, there has been no outbreak of cholera. Now, we have in this instance an experiment performed by nature on a scale large enough to serve as an absolute and exact one. This water had been contaminated with choleraic evacuations, and of course with the comma-bacilli, and it was used extensively by many human beings for several weeks. If, to speak with Koch, the comma-bacilli were the cause and essence of cholera, how is it that not one person among so many has, until the middle of December, contracted the disease? Clearly because the water did not contain the cholera virus, and because this latter has nothing to do with the comma-bacilli" (p. 36).

Other instances are cited of the occurrence of comma-bacilli in tanks wholly unassociated with any recent outbreak of cholera, and, taken altogether, the evidence adduced is strikingly opposed to the correctness of the interpretation which Koch had proposed as to the connection of the disease with water of this character, unless, as a result of seasonal and other influences, the comma-shaped organisms found by Dr. Klein in November were different from those found by Dr. Koch in February. As it is not explicitly stated that Drs. Klein and Gibbes subjected the "commas" from these tanks to the test of cultivation, it could not be accepted as established that they were identical with those of cholera; but, on the contrary, they may have been identical with those discovered by Dr. Koch in some water near the Salt-water Lakes (a few miles out of Calcutta), which he found by cultivation to be physiologically different from the comma-shaped organism of cholera. The secretary, however, has ascertained from Dr. Klein that, although it is not distinctly stated in the report that these tank-commas were cultivated, nevertheless, as a matter of fact, they had been, and were found to be identical with Dr. Koch's choleraic commas, so that this phase of the question may be looked upon as disposed of.

13. The only other evidence which has been adduced in favour of a causal connection between this organism and the disease is that acquired by means of experiments with animals. Referring to researches of this character which were performed by the German Commission and by other observers, as well as by themselves, Drs. Klein and Gibbes say:

"When in Egypt and Calcutta, Koch performed a large number of experiments by feeding, subcutaneous and intravenous injection, as well as injection into the duodenum with rice-water stools and with pure cultivations of comma-bacilli, on rodents, carnivorous animals, and monkeys, and obtained no result, and his inquiries among the people led him to the conclusion that no case was known of a domestic animal having taken cholera, and he therefore came to the conclusion that cholera is not transmissible to the lower animals. He made, however, the observation that animals (rodents) may die of septicæmia after inoculation with rice-water stools, and that the comma-bacilli are capable of multiplication within the animals inoculated, without, however, producing cholera. Since his return to Berlin he maintained that he has been able to confirm the assertions of Nicati and Rietsch—viz. that injection of the comma-bacilli into the duodenum of dogs and guinea-pigs led to death with multiplication of the comma-bacilli, and he therefore considers it proved that the comma-bacilli are pathogenic organisms.

"A large number of experiments were performed by one of us on rodents, cats, dogs, and monkeys by feeding, by subcutaneous, intraperitoneal, and intravenous injection, and by injection into the cavity of the upper part of the small intestine of mucus flakes of the ileum of typical acute cholera, and of pure cultivations of choleraic comma-bacilli and the small straight bacilli; the results of these experiments are described in the following pages" (pp. 19, 20).

"From all these experiments it follows that neither with mucus flakes taken from the ileum of acute cases of cholera nor with stools recent and old, nor with cultivations of comma-

bacilli or small bacilli, is it possible to produce in animals (mice, rats, cats, rabbits, and monkeys) any illness, be the introduction into the system carried out by feeding, by subcutaneous injection into the jugular vein, or by injection into the cavity of the intestine" (p. 24).

14. As regards lower animals, therefore, it seems to us that it has been demonstrated that neither the alvine dejections of cholera nor cultivations of isolated comma-bacilli, obtained from such dejecta are capable of producing cholera, nor even of producing systems undoubtedly of a choleraic type. On the other hand, there is no direct experimental evidence, so far as we are aware, that cholera can be induced in man by the introduction of a pure cultivation of comma-bacilli into his system; on the contrary, it is alleged that they have been swallowed with impunity.

15. The report under consideration deals with several other phases of the cholera question, but the portions referred to in the foregoing pages appear to be the most important. The investigations described, like most others recently undertaken with a view of elucidating the etiology of cholera, may, for the most part, be characterised as an attempt to confirm or refute the doctrine that the disease is caused by a microscopic comma-shaped organism. The results of these and of others of allied nature which have been brought to the special attention of the Committee may, briefly stated, be summed up as follows:

(*a*) That comma-shaped organisms are ordinarily present in the dejections of persons suffering from cholera.

(*b*) That they are not to be found in the blood nor in any of the tissues, including the mucosa of the small intestine when the latter is examined in a fresh condition.

(*c*) That comma-shaped organisms of closely allied morphological appearances are ordinarily present in different parts of the alimentary tract in health; that they are developed to an unusual extent in some of the diseases characterised by hyper-secretion of the intestine; and that there are grounds for assuming that when any predominant form is observed, it is in great measure attributable to the nature of such secretion.

(*d*) That the comma-shaped bacilli ordinarily found in cholera do not induce that disease in the lower animals, and that there are no real grounds for assuming that they do so in man; while the circumstance that they have been found in tanks which constituted the ordinary water supply of adjacent villages unassociated with the presence of the disease goes to negative any such assumption.

16. Drs. Klein and Gibbes have made a valuable contribution to our knowledge of the bacterial organisms associated with cholera, though the evidence hitherto adduced does not warrant the conclusion that any of them bear a causative relation to the disease. As regards the question of its essential cause, the Committee are glad to learn that the Government of India are making further arrangements for having investigations, of a varied character, continuously conducted in that country under the direction of Dr. Douglas Cunningham.

17. Although the precise cause of cholera has not been ascertained, sufficient is known of the general character of the disease to serve as trustworthy basis for practical action, and the Committee feel that they ought not to separate without expressing their conviction that sanitary measures in their true sense, and sanitary measures alone, are the only trustworthy means to prevent outbreaks of the disease, and to restrain its spread and mitigate the severity when it is prevalent. Experience in Europe and in the East has shown that sanitary cordons and quarantine restrictions (under whatsoever form) are not only useless as means for arresting the progress of cholera, but positively injurious; and this not merely because of the many unavoidable hardships which their enforcement involves, but also because they tend to create alarm during periods of epidemics of the disease, and to divert public attention at other times from the necessity which constantly exists for the prosecution of sanitary measures of assured value—measures which, moreover, tend to mitigate the incidence of all forms of disease.

August 4th, 1885.

The Leeches of Japan.

By

C. O. Whitman, Ph.D.

With Plates XVII, XVIII, XIX, XX, and XXI.

PART I.

THE TEN-EYED LEECHES, OR THE HIRUDINIDÆ.

The material for a study of the Leeches of Japan, including the land, freshwater, and marine Leeches, was collected during my connection with the University of Tokio (1879—1881). The coloured drawings were executed by a young Japanese artist, Mr. Nomura, who has spared no pains in making them exact copies of the living objects. Attention to the minutest details, infinite patience, a trained eye, and a remarkably skilful brush, gave results that are certainly marvels for neatness and accuracy.

For the assistance of Mr. Nomura I am indebted to Mr. Kato, president of the University; and for this and other aid my hearty thanks and grateful appreciation are due.

The ten-eyed Leeches, embracing Hirudo, Aulostoma, Hæmopis, Macrobdella, and some other genera, form a natural division of the Leech tribe, which may, for the present, be conveniently called the Hirudinidæ. The remaining families, the Nephelidæ, Clepsinidæ, Branchellidæ, Branchiobdellidæ, will be treated in separate parts.

The part here presented embraces a description of the Land Leech, the Medicinal Leech, and three species of toothless

Leeches, which form together a new genus (Leptostoma[1]); and a comparison of a few Asiatic, European, and American forms.

A considerable portion of the paper is devoted to a comparative study of the different genera, with a view to finding a more satisfactory basis for classification than has hitherto been employed.

It has been found that all the Hirudinidæ agree in having twenty-six somites represented between the first pair of eyes and the acetabulum; and a careful study of the annular composition of the somites in different genera has revealed a law of abbreviation which holds true of both ends of the Leech. The extent of this abbreviation, which consists in the suppression of from one to four of the less important rings in the extreme somites, furnishes one of the best means for distinguishing genera and species, and at the same time gives us a key to their phylogenetic relationship.

A prominent place has been given to the Land Leech, one of the most interesting and instructive forms, and one which has hitherto received very little attention. An attempt has been made to arrive at satisfactory views respecting its origin and affinities; and some general conclusions, based on a comparative study of a considerable number of species from different countries, have been offered in advance of a monograph now in preparation, which will treat of the entire family.

The Medicinal Leech has been compared with species obtained in Saigon, Singapore, Java, Ceylon, Naples, Sweden; and the genus Hirudo defined with the precision required to make it a convenient standard of comparison.

Internal structure has been dealt with to a limited extent; and some interesting facts, especially in relation to the nephridial organs of the Land Leech, have been obtained.

One of the most important points made clear in the course of the paper is the existence of from twelve to fourteen sense-

[1] In a preliminary paper ('Proc. Amer. Acad. Arts and Sci.,' vol. xx, Sept., 1884) I have used the name Microstoma, not being aware at the time of writing that the same name was already in use.

organs on the first ring of each complete somite, and the serial homology of these with the eyes.

In a postscript I have given the results of a histological study of these sense-organs, and considered the question of their function.

The Land Leech.

The Land Leech has long been known to naturalists, but chiefly through reports of a non-scientific character. The burden of the story so often told by travellers, missionaries, and army officers returning from the East, especially from Ceylon and the Himalayas, is that the land Leech is a bloodthirsty little pest which often makes itself extremely troublesome to both man and beast. An army surgeon has reported several cases in which men have been made cripples by their bite; and an old authority, Bosc, has given wide circulation to the assertion that persons asleep have sometimes been attacked by these creatures in such numbers that death has ensued. Naturalists on collecting tours have sometimes found the woods so thickly settled by these little bloodsuckers that they could save themselves only by beating a very hasty retreat. A whole battalion of English soldiers, according to report, were once driven out of the woods by such overwhelming numbers of leeches that facing them was found to be quite impossible. They advance with such astonishing rapidity that some observers have been led to believe that they can actually spring from the ground, and have therefore given them the name of "jumping Leeches."

As an example of what has been written on this subject, the following remarks by old Robert Knox[1] are introduced:

"In dry weather none of them appear, but immediately upon the fall of rains, the grass and woods are full of them. These Leeches seize upon the legs of travellers, who, going barefoot, according to the custom of that land (Ceylon), have them hanging upon their legs in multitudes, which suck their blood till their bellies are full, and then drop off. They come

[1] 'Historical Relation of the Island of Ceylon,' pp. 48, 49, 1681.

in such quantities that the people cannot pull them off so fast as they crawl on; the blood runs pouring down their legs all the way they go, and it is no little smart neither; so that they would willingly be without them if they could, especially those that have sores on their legs; for they all gather to the sore."

The tales of bloody encounters narrated by Hooker, Hoffmeister, Semper, and many others, have given the Land Leech an odious reputation; and such accounts are responsible for the prevailing notion that the creature is repulsive in appearance as well as fierce in behaviour. The brief accounts that have appeared since the time of Knox, not including the more recent descriptions by Tennent, Schmarda, and Grube, have nearly all come from persons who knew the Land Leech only as a loathsome pest. From the popular standpoint, it must be admitted that the character of the Land Leech has little to recommend it and much that is calculated to inspire disgust. On the other hand, from the standpoint of the zoologist, it may be said that no other member of the whole class of Leeches can lay higher claims to our attention and interest. Myriads of these Leeches are certainly able to give the intruder into their haunts a reception that would leave a lasting, and very likely a painful, impression. But when we reflect that the very traits which render them so irresistible in attack and so offensive in character, furnish unmistakable evidence of the severity with which their energies have been taxed in the struggle for existence; and that by virtue of these traits they have been preserved and have far outstripped their nearest relative, the freshwater Hirudo, we are prepared to admit that their energy, voracity, pertinacity, dexterity, and swiftness in attack call for admiration rather than disgust, and that their peculiarities of structure, mode of life, geographical distribution, and ætiology are subjects quite worthy of careful study.

The geographical distribution of the Land Leeches, of which about a dozen species are known to me, is somewhat more limited than that of the Land Planarians. They are found in abundance in the lower ranges of the Himalayas, where the upper limit of their vertical distribution is said to be not less

than 11,000 feet above the level of the sea (Hooker). They are very common in the damp hilly districts of Ceylon, where, according to Schmarda, they become less numerous at elevations above 4000 feet. Thunberg and A. B. Meyer have met with them on the slopes of Java; and Dr. C. Ph. Sluiter has also collected them in this island, and has furnished me with specimens obtained on the Preanger mountains and in the neighbourhood of Batavia. Marsden found them in Sumatra; Meyen, Semper, and Meyer, in Luzon; Semper, in Mindanao and the Pelew Islands; Knorr, in Japan; Meyer, in New Guinea, and Celebes (Minahassa); Mr. Haswell, in New South Wales and Queensland; and Gay and Philippi, in the southern provinces of Chili. Mr. Jijima and Mr. Sasaki found them very abundant on mountains in the central part of Japan; and I have collected them on a mountain near the eastern coast (Suberi-yama, Hakone). Through Mr. Trimen and Mr. Ward I obtained specimens of the Singhalese species from the botanical garden at Peradeniya, near Kandy; and I collected large numbers of them in the plains, at a place called Kesbawa, about twelve miles from Colombo. Quite recently Kennel has reported a Land Leech from Trinidad.

Although the stray notices and remarks on the Land Leech that have appeared since the time of Knox are sufficiently numerous to make a good-sized volume, only the few pages written by Emerson Tennent,[1] Ludwig Schmarda,[2] and Ed. Grube,[3] have any permanent scientific value. Only two species have been described, and I find no allusion to the Japanese species beyond the simple statement of Grube (2 *a*, p. 59) that it was seen by Knorr.

[1] Tennent, (*a*) 'Ceylon,' 4th edition, i, pp. 302—5, London, 1860. (*b*) 'The Natural History of Ceylon,' pp. 479—483, London, 1861.

[2] Schmarda, 'Neue Wirbellose Thiere,' &c., i, 2nd half, Leipzig, 1861.

[3] Grube (*a*) "Landblutegeln aus Südaustralien," 'Jahresbericht der Schlesischen Gesellschaft,' p. 66, 1865. (*b*) "Landblutegel," same 'Jahresbericht,' p. 59, 1856. (*c*) "Anneliden,' 'Reise der Oesterreichischen Fregatte Novara um die Erde in den Jahren 1857—59,' 'Zool. Abth.,' 3, vol. ii, p. 41, Wien, 1868.

HÆMADIPSA, Tennent (1861).

Hæmadipsa, Tennent, 1861. Hæmopis, Schmarda, 1861. Chthonobdella, Grube, 1865, 1868.

The older authors, Bosc, Blainville, Moquin-Tandon, &c., included the Land Leeches in the genus of freshwater leeches, known as Hirudo since the time of Ray. Tennent was the first to introduce a new generic name for the Land Leeches of Ceylon. Schmarda, who claims, erroneously as I believe, that there are several different species in Ceylon, refers them doubtfully to the genus Hæmopis. Diesing follows Schmarda in this respect. Grube was led by comparison of a few species to see the propriety of establishing a new genus, and—evidently in ignorance of Tennent's work—proposed the name Chthonobdella. This name is unquestionably better chosen than Hæmadipsa, but the claim of priority makes it necessary to abide by the latter.

All the Land Leeches cannot well be included in the same genus, as will be shown more fully in a later paper embracing all the species at present known. The Australian species, for which I am indebted to Mr. Haswell, differs from all the other species that I have thus far examined in having only two jaws. The latero-ventral jaws are present, but the median dorsal jaw is entirely absent. This remarkable distinction, taken together with the fact that the genital orifices are separated by seven and a half rings instead of five, as in the case of most other Land Leeches, seems to make necessary the establishment of a new genus, for which I propose the name Geobdella.[1] Hæmadipsa may be reserved for the species found in Ceylon, India, Japan, &c., which have three jaws and five rings between the genital apertures. This genus may be more fully characterised as follows:

Terrestrial. Body, at rest, 2—3 cm. in length, sub-cylindrical, tapering slightly forwards; cephalic lobe, at rest, rounded,

[1] The fact that this name, once applied by Blainville to Trocheta, has now been entirely superseded by the latter name, removes any serious objection to its use here.

but pointed in extension; acetabulum moderately large, round or oval, often obtusely acuminate in front, centrally attached, separated from the body only by a feeble constriction; ocelli in five pairs, the rings bearing the third and the fourth pair not separated by an intervening ring as in Hirudo; the rings bearing the fourth and the fifth pairs separated by two rings; œsophagus with three plications, one dorsal and two latero-ventral; maxillæ three, armed with numerous denticles that increase in size towards the converging anterior ends of the jaws, and curve slightly in the opposite direction; clitellum includes fifteen rings (= three somites); genital orifices separated by five rings; nephridial pores situated in the margin of the body, instead of on the ventral surface, the last pair opening in the constriction that separates the acetabulum from the body, and marked by three minute over-arching lobes which are usually paler in color than the rest of the body; segmental papillæ above and below, strongly developed on the dorsal side.

HÆMADIPSA JAPONICA,[1] nov. sp., Pl. XVII, figs. 1—7.

Diagnostic Characters.

Body, in extension, nearly cylindrical, tapering gradually towards the head (figs. 3, 5), about 5 mm. in diameter near the acetabulum, and 2 mm. just behind the cephalic lobe; at rest, more flattened, resembling Hirudo in shape (fig. 1). Length, at rest, 20 mm.; in extension, 50 mm.

Cephalic lobe, in extension, very pointed (figs. 3, 5) at rest, rounded (figs. 1, 2).

Acetabulum 6—7 mm. in diameter; circular, or ovato-rotundate, with the narrower anterior end very obtusely acuminate, as in figs. 5 and 9; centrally attached.

Annuli 96.—The first three, bearing the first, second, and third pair of eyes, are obscurely marked; the fourth and fifth

[1] This is not Hirudo japonica auctorum, a name which I have been compelled to ignore, since the meagreness of the description makes identification impossible.

coalesce on the ventral side, and the sixth and seventh are less deeply separated on the ventral than on the dorsal side. The anterior portion of the body generally appears more deeply annulated in extension than at rest.

Buccal Annuli—the fourth and fifth, which unite to form a single ring on the ventral side. They form the lateral and ventral boundary of the buccal aperture.

Genital Apertures.—The male orifice lies between the 29th and the 30th ring, or between the 25th and the 26th, if we begin with the buccal rings and count them as a single ring, as they appear when seen from the ventral side.

The female orifice lies between the 34th and 35th, or 30th and 31st, counting on the ventral side.

Clitellum embraces fifteen rings (three somites), beginning with the 25th and ending with the 39th. These rings have sometimes a dusky hue.

Anus, behind the last annulus, between this and the posterior sucker.

Ocelli, five pairs; the first four pairs arranged in a semicircle on the first four rings, the fifth pair on the 7th ring. The absence of a ring between the rings bearing the third and fourth pairs of eyes is a character in which all the Land Leeches agree, and one which distinguishes them from Hirudo, Hæmopis, and Aulostoma.

Œsophagus has three folds, one median dorsal, and two latero-ventral.

Maxillæ three, corresponding in position with the three œsophageal folds; relatively larger, higher, and thinner than in Hirudo; armed with about ninety denticles, which increase in size in the direction of convergence of the jaws, and curve slightly in the opposite direction. It is thus the inner posterior end of the jaw, which here, as in the Medicinal Leeches, is furnished with the larger denticles. The scar has the form of three converging lines forming about equal angles with one another, precisely as in Hirudo.

Nephridial pores open in the marginal line of the body instead of on the ventral side, as in Hirudo. There are seven-

teen pairs located in the following rings :—12, 17, 22, 27, 32, 37, 42, 47, 52, 57, 62, 67, 72, 77, 82, 87, and 93. Their position is in the hind edge of the ring which precedes the ring bearing the segmental papillæ, as will be seen from fig. 7. The successive pairs are thus five rings apart, with the exception of the last pair. The last pair lie under the anterior of three peculiar lobe-like extensions of rings 93, 94, and 95. These marginal lobes, of which the middle pair are the smaller, are flattened, project obliquely backwards, and rest on the upper surface of the acetabulum. The area immediately surrounding them, as well as the lobes themselves, is paler than the rest of the body.

Segmental Papillæ.—There are twenty rings which, with the exception of the first and the last, project slightly beyond the others (figs. 3, 5, 6, 7) ; and as each (first and last excepted) bears six dorsal and six ventral papillæ, they may be called the papillate rings. The order of these rings, which begin with the 4th and end with the 93rd ring, is as follows :—4, 7, 10, 13, 18, 23, 28, 33, 38, 43, 48, 53, 58, 63, 68, 73, 78, 83, 88, 93. The papillæ are thus borne by every fifth ring except in the anterior portion of the body, where, owing to a reduction in the number of rings to a somite, they occur on every third ring.

Colour.—The dorsal surface is divided into three longitudinal areas, a median and two lateral. The median area is always lighter in colour, and slightly wider than the lateral areas. Two dark brown stripes form the boundary lines between the median area and the lateral areas, and a third dark brown median stripe divides the median area into two halves. The median and wider stripe usually extends from between the first pair of eyes to the anus, and is of nearly uniform width throughout; but in some cases (figs. 6 and 7) it vanishes, or nearly so, on the cephalic end and on a few of the posterior rings. The lateral stripes usually vanish four to six rings behind the fifth pair of eyes, and seldom reach quite back to the posterior sucker. They are besides somewhat more irregular in outline than the median stripe.

There are two pale yellow marginal stripes which vanish before reaching the head, and end rather abruptly behind four or five rings in advance of the rings whose margins are produced into the lobes above described.

The median area is most often a dull yellowish brown, considerably lighter than the lateral areas. In some cases (figs. 1, 6, 7) the median area inclines rather to a reddish hue, the lateral areas being a deeper and richer shade of the same colour. The lateral areas and the somewhat lighter ventral surface are generally sprinkled with fine specks of dark brown. Sometimes, as seen in fig. 5, a feeble tinge of olive is perceptible. The anterior border of the cephalic lobe has a smoky hue which frequently extends to the entire head and anterior portion of the body. The acetabulum is a very pale green or olive above, and smoky brown or olive brown below.

The clitellum is sometimes marked by a deeper shade than the rest of the body (fig. 5).

The ground colour of the Singhalese species (figs. 8, 9) is a rich reddish brown flecked with dark brown, more profusely above than below. The head and posterior sucker show the smoky hue seen in the Japanese species. There are no dark stripes, and, as a rule, no indications of a median area. There are two marginal stripes and one median, all of which are a bright lemon yellow.

Among several hundred specimens I found two or three in which a lighted median area was more or less imperfectly indicated. In all other particulars this species agrees completely with Hæmadipsa japonica.

Habitat.—So far as could be ascertained, the Japanese Land Leech is confined to mountain slopes and ravines, never descending into the low plains, for which reason the Japanese call it the Mountain Leech ("Yamabiru:" yama, mountain, and hiru leech). Specimens were collected by Mr. Jijima from Akihazan, a mountain about 4000 feet high, situated near the centre of the province of Totomi (Enshiū), by Mr. Sasaki on mountains in the provinces of Mino and Iga, between 34° and 36° lat., and by myself on a mountain in

Hakone, called Suberiyama. They are also said to be found on Amaki peak, in the province of Idzu, on the eastern shore.

Habits.—I have never seen the Land Leech of Japan on trees, and I believe it keeps itself habitually on the ground, in the moss, or under damp leaves and loose rubbish. When awakened by the footsteps of man or beast, it quickly appears on the surface, and frequently ascends low plants and occasionally perhaps trees in search of the intruder. They are usually found near the tops of mountains, in damp ravines or dense thickets, where the ground is carpeted with moss and other low plants. During the driest months of the summer these localities are kept moist by mists and showers, and in winter they are sometimes covered with snow. Wild boar and deer frequent these places, and it may well be that these Leeches derive their sustenance in part from such animals. They are much dreaded by the natives, who are accustomed to go with feet and legs bare. They are extremely voracious, and wonderfully rapid in their movements. When once on the person, they take such rapid strides, and cleave with such pertinacity, that it is difficult to remove them without injury. Their bite is so gently executed that it would hardly be felt unless the attention were specially directed to it; but the wound is comparatively deep and the scar often remains for months. They gorge themselves with blood in thirty or forty minutes, and then drop off. During the process a transparent liquid exudes from the skin, which keeps moist both the Leech and the object on which it preys, and even flows away in a few large clear drops. It would not be difficult with a dozen Leeches to collect enough of this fluid for chemical analysis; but I neglected to do this. I think the fluid comes in part from the mucous glands of the skin, and in part from the nephridia. If the moisture be removed by a momentary application of blotting-paper, it is easy to see, on removing the paper, the fluid gathering over the nephridial pores. When the Leech creeps over a dry object it leaves a slimy path similar to that left by land snails. When fully gorged with blood they become sluggish, and do not appear to be averse to going

into water; at least all the specimens which I have fed retired to wet moss and lay wholly or partially covered with water. Hungry specimens confined in a bottle containing a little water remain always above its surface. If dropped into water they do not swim like aquatic Leeches, but sink to the bottom and then creep out again. They are often found in the neighbourhood of small streams, but never in them. Although they have such a decided preference for terrestrial life that they probably never visit the water, even when it is within easy reach, they have not lost the power of living in it for at least a considerable time. One of the Singhalese specimens was kept in water thirty days, and this long submersion resulted in no perceptible injury.

It is interesting to watch the behaviour of hungry specimens confined in a bottle which is kept moist by wet moss at the bottom. They are very quiet so long as the containing vessel iis left undisturbed, but they are very sensitive to any sudden jar or quick movement of the air. They appear to avoid the light and to seek the side least exposed to it. At rest the head and anterior half of the body are often raised as if held in readiness for the attack. If the bottle is opened and a puff of breath blown upon them, they are instantly thrown into a state of great excitement; after a few hasty reaches in different directions have convinced them that the disturber is not in immediate reach, they begin to ascend; and the foremost among them, reaching the rim of the bottle, halt for a moment, standing quite erect and extended as if hesitating in which direction to advance; another puff or a slight jar sets them again in commotion, and they swing to and fro, reaching in all directions for the object of their search. If one attempts to put them back he finds them more than a match; for while trying to thrust one back a dozen others rush on to the hand, and in a few moments are scattered over the body. The best mode of recapturing them is to place over them an inverted bottle, into which they will ascend.

In collecting it is best to use a deep bottle, and to take advantage of their disposition to ascend by keeping it inverted;

for then they will be induced to take a position as far as possible from the mouth, and new specimens may be added without giving those already in time to escape.

In moving about, the cephalic lobe is much elongated, and its obtusely pointed tip appears to be used as an organ of touch. The annuli of the anterior portion of the body are at the same time more prominent and the eyes more protuberant. The mode of progression is the creeping movement common to all Leeches. The head is thrown forward as far as the extended body will permit, and the oral sucker having been fixed the body is drawn up into a vertical loop, and the posterior sucker placed close to the anterior. These looped strides may be repeated with such rapidity as to give the appearance of jumping, but such a movement is plainly impossible in this mode of locomotion.

Comparison of the Land Leech with the Medicinal Leech.—A comparison of the Land Leech with the Japanese Medicinal Leech, which agrees in all its leading features with the continental varieties of Hirudo, affords unmistakable evidence of genetic relationship between the two genera.

In both the land and the aquatic Leech we find that the typical somite embraces five rings; but the two species show a difference of at least five in the total number of rings composing the body. This fact might lead one to suspect that the Land Leech had lost an entire somite; but a careful study of the two cases does not support this view. In Pl. XVIII, fig. 10, I have represented the whole number of rings in the Japanese Medicinal Leech. The total number of rings may be made to vary, apparently at least, according to the mode of counting. Most authors count the rings as they are seen on the ventral side, beginning with the buccal ring (5th and 6th in my figure), and take no account of the fragmentary post-anal ring; thus counted there would be but ninety-five rings, which is the number usually given for H. medicinalis of Europe. Again, if the rings are counted from the dorsal side, leaving the ventral aspect entirely out of consideration, we find that the buccal and post-buccal are each double, and must be counted

as four instead of two. This gives an increase of two rings, whcih, added to the four cephalic rings and the post-anal, gives a total of 102. In a close comparison like the one we are about to make, it will soon be seen that both the dorsal and ventral aspects of the rings must be considered, and that it is advisable to include in our count the rings of the cephalic lobe which are not seen from the ventral side. We are now prepared to take up the comparison of rings with a view to ascertaining precisely which rings have been lost by the Land Leech.

There is a universal tendency among Leeches to a reduction of the number of rings in the somites at both extremities of the body. A glance at the arrangement of the eyes and the segmental papillæ in fig. 10 makes it perfectly evident that the metameric division extends to the very end of the cephalic lobe. The 1st and 2nd somites are each represented by a single ring bearing a single pair of eyes; the 3rd somite has two rings, the first of which bears the third pair of eyes; the 4th, 5th, and 6th somites include each three rings, the fourth and fifth pairs of eyes being borne on the first rings of the 4th and 5th somites. Behind there are three somites of two rings each and then a somite of three rings. The remaining somites have each five rings. In fig. 6, in which the relation of the eyes to the segmental papillæ is very satisfactorily shown, we find exactly the same number of abbreviated somites as in the corresponding portion of the Medicinal Leech; and the reduction in the number of rings is precisely the same, except that one ring is missing in the 3rd somite, in consequence of which the third and fourth pairs of eyes are on contiguous rings instead of being separated by a single intervening ring as in fig. 10. The cephalic lobe has simply lost a single ring, which bore no eyes, and which could therefore be dropped to the advantage of those possessing a higher functional value, since the fourth pair of eyes could thus be added to the semicircle of the more important eyes. In dropping this ring the Land Leech has advanced one step in the well-trodden path of development pursued by its aquatic progenitors. The course of progress may be briefly defined as

centripetal abbreviation, the maximum limit of abbreviation or concentration appearing first of all in the most extreme somites, and advancing from these, step by step, to those that lie successively nearer the middle of the body. Allowing that abbreviation progresses centripetally, it is easy to see what ring, if any, is destined to disappear next. The next step for the Medicinal Leech is to drop the eyeless 4th ring; and for the Land Leech, first the anterior and then the posterior eyeless ring now separating the fourth and fifth pairs of eyes.

That every step thus far taken in this direction has been beneficial, appears evident enough from the fact that the eye-bearing rings have been retained and functionally improved in proportion to the number of the less important eyeless rings sacrificed. These rings have been still further advanced by transverse concentration, the more important elements being brought into closer order and strengthened at the expense of the parts eliminated. The 1st ring in the Land Leech, leaving out of consideration the thin, lighter-coloured margin, is represented by two large eye-bearing plates; the 2nd by two slightly smaller eye-bearing areas and two still smaller median areas, bearing segmental papillæ, or incipient eye-spots; the 3rd, by two similar ocellated areas and eight small interposed areas; the 4th, by two still smaller ocellated areas and six small intermediate areas; and the 7th, by a row of small areas in two of which are seen the posterior pair of eyes, which are considerably smaller than those of the preceding rings. The only incongruity in all this with the view here taken lies in the fact that the third ring has a larger number of intermediate plates or areas than the fourth, which is the reverse of what we might have expected. The arrangement of these areas, however, suggests an explanation of the difficulty. They form a single transverse row which becomes double at the two ends adjoining the ocellated areas. The most natural way of accounting for this duplicity is to assume that two of these areas are remnants of the ring that has disappeared between the third and the fourth pair of eyes.

With respect to the three somites which follow the three cephalic somites, it should be noticed that, although the number of rings in each is the same, they do not exhibit the same degree of concentration and development. The 4th somite is represented on the ventral side by the coalesced buccals and the 6th ring; and on the dorsal side the first buccal (4th ring) is composed of fewer areas than the succeeding rings. The difference between the 5th and 6th somite is slight on the ventral side, but well marked on the dorsal side by the presence of the fifth pair of eyes in the former. Thus the head and anterior end of the body of the Land Leech, especially in comparison with the corresponding portions of the Medicinal Leech, plainly illustrate an order of events which may be called the law of centripetal abbreviation; and at the same time they show a strict correlation between the grade of development and specialization and the degree of abbreviation.

Remembering that the 4th ring of the Medicinal Leech is wanting in the Land Leech, it becomes very easy to identify the rings of the latter with those of the former, and to see that the sexual orifices are situated between homologous rings in the two cases.

Comparing now the hind end of the body of the Land Leech with that of the Medicinal Leech, we find that the direction of abbreviation is here also centripetal. In fig. 10 we find twenty-six somites, of which six anterior and four posterior are abbreviated; while in the Land Leech there are, apparently, only twenty-three somites, of which six anterior and one posterior are abbreviated. In both cases then there are six abridged somites followed by sixteen unabridged; and this leaves only four rings in the Land Leech to offset nine in the Medicinal Leech. There can be but little doubt that the first twenty-three somites correspond in the two species; and this being assumed, we may inquire how far the remaining posterior rings can be identified. The four posterior rings of the Land Leech (fig. 7) appear at first sight to represent a single somite; but this view is rendered doubtful by the fact that no somite of four rings occurs in Hirudo. An examination of a large number of Land

Leeches has enabled me to identify at least three of the four rings. I find that segmental papillæ are sometimes quite distinct, not only on the 93rd ring, as shown in the figure, but also on the 94th and 95th. I have not detected any satisfactory traces of these papillæ on the 96th ring, which is the last and most rudimentary of all the rings. The discovery of papillæ on the 94th and 95th, not only in this species, but also in the Singhalese and the Australian species, makes it certain that the four posterior rings do not represent one somite, but at least three, which would raise the total number to twenty-five. As the 93rd, 94th, and 95th rings each represent a somite, it is more than probable that the 96th ring represents a remnant of the papillate ring of the 26th somite. The rings may then be identified as follows:

93rd	ring	(Land Leech)	=	94th	(Medicinal Leech).
94th	„	„	=	97th	„
95th	„	„	=	99th	„
96th	„	„	=	101st	„

Thus five rings have been lost behind and only one in front. The loss at the anterior end is correlated with a higher development of the sense-organs; at the opposite end, with the enlargement of the acetabulum and the hind end of the body. At both extremities the sacrifice of rings have been restricted to the less important; and it is plain that the less specialized rings of the hind end have been the first to disappear. It is the hind end of the body that has undergone the greater changes in adaptation to life on land.

In abandoning aquatic life, the Land Leech became restricted to one of the two modes of locomotion open to it while living in the water; henceforth the practice of swimming was discontinued, while that of creeping was enormously increased to meet the requirements of the new conditions of life. The result was that the ability to swim was finally completely lost, while that of creeping was immensely improved. Adaptive changes in size, form, and proportions advanced *pari passu* with the cultivation of one mode of locomotion to the exclusion of the other. The centre of gravity travelled backward

from the central position required for maintaining the equilibrium in swimming to a point nearer the posterior sucker, keeping pace with the gradual concentration of muscular power in the sucker and posterior end of the body. The body became more cylindrical, the acetabulum and the posterior extremity stouter, thus enabling the Leech to poise on this end with great ease when reaching about for its victim.

The Segmental Papillæ.—In the foregoing comparison of the rings and somites of the land and the aquatic Leeches, attention was called to the position of the eyes and the segmental papillæ; and this leads us to a point of considerable importance, namely, the significance of the papillæ.

In the Land Leech, the epidermis is broken up into quadrangular and polygonal areas; and the larger areas are the seats of the eyes and the papillæ. The number and arrangement of these areas on the cephalic lobe are very regular and uniform in different individuals of the species. Behind the head the areas are arranged in transverse rows corresponding in diameter to the thickness of the rings. This division into areas extends to every part of the Leech, and gives the surface that rough appearance which Grube has described as "granular." In addition to the segmental papillæ, which, from their size and metameric arrangement, are very conspicuous (figs. 6 and 7), there are numerous smaller papillæ which amount to only slight rounded elevations situated at the centre of the areas which are not occupied by the eyes or the segmental papillæ. In the posterior region of the body the segmental papillæ are conical in form, with rounded summits which are pale yellowish white and translucent. At the centre of the summit there may be seen a minute dot of a plumbeous hue, which has the appearance of a pore. Sections show that there is no pore, and that the dot is merely a minute unpigmented portion of the solid papilla. Towards the head the papillæ become more and more flattened; but their lighter colour and the larger size of the lead-coloured central dots make them quite distinct. Owing to the bilaterally symmetrical arrangement of these papillæ on the first ring of each somite, there are as

many transverse rows as somites, and as many longitudinal rows as papillæ in a single ring. The longitudinal rows may be designated according to position, as median and lateral. The two median rows are the most prominent, and are placed somewhat nearer the lateral dark-brown stripes than the median stripe ; the two inner lateral rows are located just outside the lateral stripes, and the two outer lateral rows just inside the marginal yellow stripes. Thus each of the three broad longitudinal areas of colour is marked by two rows of papillæ.

In the aquatic Leech (figs. 10, 11, 18) we find six rows of spots which are plainly homologous with the segmental papillæ of the Land Leech, although smaller and only slightly raised into papilla-like protuberances.

On the ventral side of both the land and the aquatic Leech are also found six rows of these segmental papillæ or spots ; but here they are so feebly developed that they might be overlooked. They are placed on the rings that bear the dorsal rows and are similarly disposed.

These segmental spots have been described in various species of aquatic Leeches, but no one has hitherto studied their structure, or offered even a plausible suggestion as to their function. Their arrangement on the dorsal side, as shown in fig. 6, suggests an explanation of their nature, which is corroborated by a study of their histological structure. It is perfectly plain that the fifth pair of eyes occupy the places of two of these spots in the inner lateral rows. It is also easy to trace the median rows into the first pair of eyes. As will be shown more fully in describing the Medicinal Leech, the first pair of eyes must be genetically associated with the two median rows of segmental papillæ; and all the remaining eyes with the two inner lateral rows. According to this view, the eyes and segmental papillæ were, primarily, morphological as well as physiological equivalents ; but this does not necessarily imply that they now have the same functional significance. The original segmental papillæ may have represented sense-organs of a more or less indifferent order, among which, in

the course of the historical development of the Leech, a division of labour was introduced, a few at the anterior extremity becoming specialised as organs of vision, the rest either remaining in their early indifferent condition or becoming specialised in some other direction.

It seems more probable, however, that the segmental papillæ are incipient eye-spots—visual organs in statu nascendi—and that the eyes are organs of the same nature, only structurally improved and functionally exalted.

The Structure of the Eyes and Segmental Papillæ.—If any such relationship exists between the eyes and the segmental papillæ as is indicated by their correspondence in position, we should expect to find some important resemblances in their structure and composition. As this subject will be considered in detail in a later paper provided with illustrations, I shall here call attention only to the more important points.

The eye of the Land Leech, like that of the aquatic Leech, is formed of large clear cells ("eigenartige helle Zellenkörper," of Leydig), which are usually regarded as a corpus vitreum, surrounded by a thick layer of pigmented cells. The epidermal layer covering the eye is composed of closely packed columnar cells, which are not perpendicular to, but inclined towards the centre of, the convex outer surface of the eye. This epidermal cap is further distinguished from the epidermis elsewhere in being entirely free from pigment. The cell nature of the large clear bodies forming the central portion of the eye has been denied by Ranke, on the ground that no nuclei had been discovered in them by himself or other authors. My sections, however, demonstrate the existence of nuclei in these bodies. The nuclei are extremely small and usually situated very near the outer side of the cells, close to the pigmented layer, and are therefore easily overlooked. Within each of these clear cells there is found a very peculiar white corpuscle which never stains. Leydig represents this body in a few cases as a complete ring, but in most cells as an imperfect ring opening towards the base of the eye. In the

Land Leech this body is band-shaped and bent in various directions, so that in section it often appears to consist of several separate pieces, which may be straight, bent, or looped. The optic nerve does not enter the eye at its base, but at some little distance from the base on the anterior side.

The clear central cells of the eyes are very remarkable elements, differing in their general appearance and structure very conspicuously from any cells that have hitherto been discovered in other parts or organs of the Leech. But I have found that these peculiar cells—from two to four or more in number—are also present in each of the segmental papillæ of the ventral as well as of the dorsal side, in both land and aquatic Leeches. I have succeeded in tracing a nerve up to these cells, without, however, finding any connection. As before stated, the lead-coloured dots at the centres of the segmental papillæ are free from pigment and transparent like the epidermal cap of the eye. The only important difference in composition between the eyes and the papillæ is the absence of the pigment layer in the latter. This difference is not easily reconciled with the view that these papillæ are ocular in character. Still the fact that they are much larger on the dorsal than on the ventral side, and the presence of those "peculiar (sense ?) cells" situated just below a window-like opening in the surface pigment, as well as their obvious serial relationship with the eyes, favour such an interpretation. The evidence pointing in this direction is, perhaps, somewhat weakened by the fact that those same clear cells, which have hitherto been regarded as peculiar to the eyes, are found alongside the nerves running to the "goblet-shaped" sense-organs located in the margin of the cephalic lobe. The presence of these cells in the segmental papillæ cannot therefore decide the question of their physiological significance. It is quite certain, however, that these papillæ are not respiratory organs, as suggested by Ebrard. Their position is not in favour of their being organs of taste or smell; and their structure is opposed to the idea that they are either auditory or tactile organs.

The distribution of the large clear cells, each with its enigmatical band-shaped corpuscle and minute nucleus, among the different sense-organs, appears to show that they are sense-cells, and to throw considerable doubt on the commonly received opinion that they function merely as a corpus vitreum in the eye of the Leech. The fact that the optic nerve, after penetrating the eye, can be traced for some distance along its axis between these cells is in itself sufficient evidence that they cannot be explained as a purely dioptric apparatus (cf. Postscript).

The Nephridia.—The nephridial organs agree in the main with those of the Medicinal Leech, which have been so well described by Bourne,[1] but differ from them in three important particulars. 1. The efferent ducts terminate in the margin of the body, instead of on the ventral surface at some distance from the margin. 2. The vesicles are much larger than in the aquatic Leech. 3. The three pairs of vesicles located within the region of the clitellum are lined with very thick cubical cells which form irregular folds, projecting into the cavity, while they are elsewhere lined with thin pavement epithelium.

In the European Hirudo, the vesicles are oval sacs, the larger diameter of which is only about twice that of the testicular sacs, and are located just outside the vasa deferentia, the successive pairs alternating with the testes. In the Land Leech the vesicles are capacious sacs holding the same serial relation with the testes, and lying partly beneath, but mainly external to, the cæca of the stomach. As they are opposite, and continuous with, the cæca, their shape conforms in the main to that of these appendages, and hence must vary according to the degree of distension of the latter. In a horizontal section of the Leech, one of these vesicles is seen to extend in an antero-posterior direction through from three and a half to four rings; while in Hirudo it bridges only two rings, less than

[1] A. G. Bourne, (*a*) "On the Structure of the Nephridia of the Medicinal Leech," 'Quart. Journ. Mic. Sci.,' xx, July, 1880, p. 283. (*b*) "The Central Duct of the Leech's Nephridium," idem., vol. xxii, July, 1882, p. 337.

half the somite. In a transverse direction the vesicle has about the same extent, so that its capacity is well nigh equal to that of the undistended cæcum.

It is apparent then that the vesicle here represents a bladder-like reservoir, the capacity of which, relatively speaking, must at the lowest estimate be more than double that of the corresponding part in the Medicinal Leech. I have not discovered any cilia in the vesicle, but I am not prepared to say that they are wanting.

The efferent duct is composed of two distinct portions; the lumen of the inner portion is much larger than that of the outer, and is lined with an epithelium quite like that of the vesicle; the outer portion, which is nearly equal in length to the inner, is lined by an involution of the epidermis, and is supplied with both ring and radial muscle-fibres. The inner portion is furnished with ring fibres alone, which are multiplied in number at its junction with the vesicle, so as to form a powerful sphincter. The course of the comparatively long efferent duct is nearly at right angles to the axis of the body, the inner portion being nearly horizontal, and the outer inclining a little upward to reach the margin.

The glandular part of the nephridium is somewhat larger relatively than in Hirudo, lies in front of the vesicle, and opens into it by a funnel-shaped orifice. The "vesicle duct" passes directly into the smaller "central duct," which, after perforating a convoluted chain of shells, enters the more massive portion in which the cells are arranged radially. According to Bourne, the vesicle duct in Hirudo is "formed by numerous cells, several cells surrounding the lumen of the tube." In the case of the Land Leech the vesicle duct is formed of a single chain of cylindrical cells, each cell entirely surrounding the lumen. The chief difference then between this duct and the adjoining portion of the central duct is its greater lumen.

It remains to find some explanation for the extraordinary size of the nephridial vesicles. It is now generally admitted that the nephridia are renal organs; and this view of their function has tended to bring into discredit the idea that the

liquid secretion of these organs serves any useful end in the economy of the Leech.

Moquin-Tandon[1] designates the vesicles as "poches de la mucosité;" and after alluding to the old belief that they were organs of respiration (Schlacht, Bibiena, Thomas, Dugès and Audouin), states that "on les regarde aujourd'hui, avec raison, comme réservoirs de mucosité."

Ébrard,[2] who gives a detailed account of the formation and deposit of the egg-case, claims that the superficial portion of this capsule, which has a spongy texture, is formed from the secretion of the nephridial organs ("anses mucipares") which lie before and behind the clitellum, while the internal portion is the product of the subcutaneous glands of the clitellum. Ébrard thus regards the nephridia as organs of secretion comparable to the colleterial glands of insects ("gland sérifique") and bases this view on the following observations:

"Ayant ouvert, ai-je déjà dit, une Sangsue qui se disposait à poser un cocon et qui commençait à former de l'écume, je trouvai que toutes les poches de la mucosité étaient très-dilatées et remplies de liquide. Chez une autre Sangsue, au contraire, que j'ouvris alors qu'elle était entourée d'écume de toutes parts et immobile, les poches de la mucosité étaient toutes vides, sauf celles de la ceinture. On reconnaissait qu'elles venaient d'être distendues. Je me crois donc autorisé par ces observations, à penser que le liquide mucilagineux qui, agité par la tête de la Sansgue, se convertit en écume puis se change en tissu spongieux, est secrété par les orifices des poches de la mucosité."[3]

The fluid enclosed along with the eggs in the capsule is supposed by the same author to come from two sources, namely, the uterus and the nephridial vesicles belonging to the region of the clitellum. That the renal fluid should have two such entirely unlike uses, sharing, on the one hand, with the secre-

[1] 'Monographie des Hirudinées,' p. 129, Paris, 1846.

[2] 'Nouvelle Monographie des Sangsues médicinales,' pp. 79, 117, 119, Paris, 1857.

[3] Loc. cit., p. 119.

tion of the gland-cells of the clitellum the work of forming the cocoon, and serving, on the other, in common with the fluid discharged from the uterus, as reserve food-material for the young, is a supposition neither probable in itself nor well supported by observation. By what means could the Leech gather the renal fluid around the clitellum? And, allowing that this could be accomplished, by what process could the fluid be converted into the spongy substance of the cocoon? That such a transformation requires some explanation is evident from the fact that the fluid does not take the form of a spongy body on other parts of the body. Leuckart[1] has shown conclusively that both the capsule and its spongy mantle are of the same chemical and physical nature; and the manner in which the cocoon is formed leaves little room to doubt that its substance is derived exclusively from the unicellular glands of the clitellum. This is the view taken by Leuckart, Lankester,[2] and, so far as I know, by all the more recent writers.

That the nephridia within the limits of the clitellum concur with the uterus in supplying the fluid contents of the cocoon, seems to me not altogether improbable, in view of the peculiarities of the vesicles of this region in the Land Leech. The only observation in favour of this opinion adduced by Ébrard is the following:—A single Leech was opened at the moment when the capsule was nearly ready for the reception of the eggs; and the vesicles within the clitellum, and within this region only, were found full of fluid; the same vesicles were found empty in another individual that had just deposited a cocoon. Perhaps we shall not show too little respect for an opinion based on a single experiment of this kind, if we venture to express a regret that Ébrard did not, so far as can be learned from his statements, repeat his observation before giving it the importance of a general fact.

In the Medicinal Leech, the fourth, fifth, and sixth pairs of vesicles lie within the sexual girdle, precisely as in the Land Leech; but their structure is the same as that of the vesicles

[1] 'Die Menschlichen Parasiten,' i, p. 684, 1863.
[2] 'Quart. Journ. Mic. Sci.,' xx, p. 304.

lying before and behind this region, and thus their morphological features neither confirm nor contradict the opinion of Ébrard.

In the Land Leech, however, we do find a strongly marked histological difference between the vesicles of the clitellum and those of the rest of the body, and this fact fully warrants the belief that they are in some way subsidiary to the reproductive organs. These three pairs of vesicles open in the 27th, 32nd, and 37th rings, and are thus clearly inside the region of the clitellum, which extends from the 25th to the 39th ring. The contents of these vesicles could easily be discharged into the cocoon, as suggested by Ébrard; but there is at least a possibility that they assist in the formation of the cocoon, and still another that their secretion aids the copulatory process, thus serving an end for which special glands have been provided in the case of Macrobdella.[1]

Under the head of "current statements as to the nephridia," Bourne, after referring to the opinion of Gratiolet and others that the nephridia are secretory, comments as follows:—"Gratiolet considers that they also serve to keep the skin moist while the animal is out of the water, and correlates the greater power the Medicinal Leech has of staying out of water compared with that of the Horse Leech with the larger size of these organs in the former animals. Leydig has shown, however, that unicellular glands open all over the surface of the skin, and these would serve to keep it moist, just as in Land Planarians, the frog, and other terrestrial animals which possess a moist skin. I see no reason to suppose that the nephridia of the Leech have any such mucous function."[2]

Moquin-Tandon and Ébrard have called attention to the fact before mentioned, that when the surface of the Leech is made uncomfortably dry by means of paper or dust, fluid may be seen to gather in small drops corresponding in position with the nephridial pores. This experiment I have often repeated with both aquatic and Land Leeches, and always with the same

[1] Leidy, 'Proc. Phil. Acad. Nat. Sci.,' p. 230.

[2] Loc. cit., p. 285.

result. We have then experimental proof that the Leech can moisten its ventral surface at least with fluid discharged from its nephridia. If the loss of moisture stimulates a Leech to expel its nephridial fluid, the most natural inference seems to be that the act is designed to restore the moisture. I can see no serious objection to the opinion that the nephridia may co-operate with the numerous gland-cells opening at the surface in keeping the skin moist; and I am unable, on any other hypothesis, to find a satisfactory explanation of the peculiar differences between the nephridia of the aquatic Leech and those of the Land Leech. These peculiarities were undoubtedly acquired in adaptation to terrestrial life—a mode of life which, under the most favorable conditions, must inevitably have taxed to the utmost any organs that could furnish moisture or serve as reservoirs. We are not therefore surprised to find the Land Leech provided with more numerous skin-glands and more capacious nephridial vesicles than its nearest aquatic relative.

Allowing that the nephridial secretion may serve the end we have indicated, and remembering that such service would most likely be required when the Leech is scouring about, it is plain that the marginal position of the nephridial pores would present some advantages over the latero-ventral position seen in Hirudo. The various attitudes assumed by the Leech while moving about are such as would favour the spreading of the secretion in all directions, over the dorsal as well as the ventral surface.

Under the head of "habits," I have mentioned that while the Land Leech is engaged in the act of sucking blood, it discharges a limpid fluid in such quantities that it rolls away in several drops. I have supposed that this fluid came from two sources, namely, the mucous gland-cells and the nephridia. Gratiolet appears to have observed precisely the same phenomenon in the Medicinal Leech; and he was of the opinion that the fluid came from the nephridia. As the gland-cells are undoubtedly active when the Leech is thus engaged, it does not seem probable that the escaping fluid contains no admixture of

their mucous secretion; still I am inclined to believe that much the larger part of it comes from the nephridia.

This brings us to the question, whether the nephridial fluid discharged while the Leech is sucking is for the most part secreted ex tempore, as supposed by Gratiolet; or furnished mainly at the expense of the fluid already secreted and held in reserve in the vesicular reservoirs. The following remarks by Gratiolet on this point are highly interesting, although there may be room for doubting their entire accuracy :—

"Lorsque les Sangsues étaient attachées à la peau et avaient déjà absorbé une certaine quantité de sang, je voyais sourdre sur les flancs de l'animal un fluide hyalin qui s'épanchait sur ses côtés et l'entourait fort exactement d'une zone liquide. La quantité de fluide augmentait à mesure que la Sangsue se remplissait de sang. Il s'écoulait par un courant continu, de petits orifices qui donnent issue aux vésicules des anses mucipares.

"Quelle était l'origine de ce fluide? Le sang de l'animal? Mais évidemment il excédait en quantité la masse entière du sang contenu dans ses vaisseaux. Il provenait évidemment d'une autre source, c'est-à-dire du sang étranger, introduit par la suction dans le tube digestif.

"Ainsi, au moment même où le sang est sucé, la Sangsue en sépare les parties les plus liquides, elle le concentre, pour accumuler en plus grande quantité ses éléments nutritifs. Or, les agents par excellence de cette concentration sont les vésicules et les anses mucipares; elles viennent donc d'une manière accessoire en aide aux fonctions digestives.

"Ce rapport est-il le seul? En aucune façon. Elles peuvent aider encore aux fonctions respiratoires en humectant la peau et par conséquent favoriser les excursions que fait un animal essentiellement aquatique dans un milieu aérien, et la faculté que les Sangsues, les Hæmopis, les Aulastomes et les Trochètes ont d'errer sur la terre, est évidemment proportionnelle au développement et à l'activité de ces appareils excréteurs.

"Ainsi, dans la Sangsue médicinale, ils sont très grands et

très vasculaires, or cet animal abandonne spontanément les eaux en plein jour. Ils sont beaucoup moins développés dans l'Aulastome qu'on ne voit guère errer sur la terre pendant le jour, mais seulement à l'aurore ou au crépuscle. . . . .

"Les Hirudinées, qui n'ont point la faculté d'arroser leur peau, n'abandonnent jamais les eaux où elles vivent; telles sont les Nephelis (Erpobdelles) et les Clepsines ou Glossiphonies, qu'on peut conserver indéfiniment dans des vases ouverts."[1]

While I fully concur in the opinion that the nephridia aid the respiratory functions by helping to keep the skin moist, I have found no satisfactory evidence that they assist the work of digestion in the manner indicated above. The quantity of fluid that escapes from the Land Leech during the process of sucking, certainly does not exceed the capacity of the nephridial vesicles, and hence I see no reason to suppose that the more watery and less nutritious portions of the imbibed blood are collected and discharged by the nephridia with a rapidity that would imply a constant current from the "stomach" to the exterior through the nephridial ducts. The vesicles are so placed with respect to the cæca of the stomach that the maximum expansion of the former is correlated with the minimum distension of the latter, and vice versâ; so that if the vesicles are full when the Leech begins to fill itself with blood, their contents would probably be expelled in slow but steady streams issuing at the nephridial pores. As the gradual distension of the cæca would seem to be quite sufficient to account for the escape of the nephridial fluid, it is probable that the muscles belonging to the walls of the vesicles would remain quite passive during the process, their activity being reserved for occasions of need such as might arise during a dry season or in the perambulations of the Leech.

If the vesicles serve the end supposed, it is evident that there must be some correspondence between their size and the

[1] 'Recherches sur l'organisation du système vasculaire dans la Sangsue médicinale et l'Aulastome vorace,' Paris, 1862, pp. 28—30. The same in 'Ann. des Sci. Nat.,' sér. 4, Zool., xvii, pp. 197—199.

power of the Leech to remain out of water; but as these are not the only organs for supplying moisture to the skin, it would be rash to conclude that all those Leeches which are provided with very small vesicles, or with none at all, are incapable of leaving their native element. It is certainly going too far to assert that Nephelis and Clepsine never leave the water, and that they may therefore be kept indefinitely in uncovered vessels.

There are seventeen pairs of nephridia as in Hirudo. The number, position, and external appearance of the reproductive organs agree closely with the same in Hirudo. The histological features of the internal organs will be dealt with in a future paper.

General Remarks.

Only a few general conclusions concerning the origin and distribution of the Land Leeches are here offered, as a fuller discussion of these questions may be best reserved for a paper which will deal with all the species at present known.

There are certain peculiarities of structure common to all the Land Leeches I have examined; such as the absence of an eyeless ring between the two rings bearing the third and fourth pairs of eyes, the marginal position of the nephridial pores, the large size of the vesicles, and the peculiar lobes which cover the posterior pair of pores. These features point to a common origin of species that are now widely separated. It is quite certain that at some period of their genealogical history they exchanged aquatic for terrestrial life. Their nearest relatives are the Medicinal Leeches (Hirudo), all of which, as is well known, are confined to fresh water. At first thought, it would seem somewhat remarkable that an animal so thoroughly adapted to aquatic life as the Medicinal Leech should be able to accommodate itself exclusively to life on land; but when we compare its habits and conditions of life with those of the Land Leech, and look more closely into the nature of the change implied in the exchange of respiratory media, we find little in the transition to excite our wonder. The Medicinal Leech has

the habit of crawling partly or wholly out of the water, when the air is so saturated with moisture that it can venture out without exposing its skin to undue desiccation. Remembering that the respiratory functions in the Leech are performed by the skin, and that, provided this is kept moist, it is capable of drawing its supply of oxygen from damp air, there is little difficulty in understanding how such an animal might become accustomed to living out of water altogether. Such a change would not lead necessarily to the immediate loss of any organs nor to the acquisition of new ones. Certain organs have been compelled to do more work in the Land Leech than they do in the aquatic Leech, and the consequence has been multiplication and enlargement. The skin-glands have become larger and more numerous, and the nephridial vesicles have expanded to bladder-like reservoirs, so that the Leech is still able to keep its dermal respiratory organ constantly moist.

The Land Leeches are mainly confined to islands and continents that lie within the tropics; but the extreme limits of their latitudinal distribution is not much less than 40° on each side of the equator. The highest parallel of N. lat. is touched in Central Japan; of S. lat. in the southern provinces of Chile. Notwithstanding this wide range in latitude, the conditions under which the different species live are remarkably uniform. From the Himalayas to Japan, from Ceylon to Chiloe, they have established themselves in localities that present exceptionally even, and almost identical, conditions of climate. Neither in the most northern nor in the most southern latitudes of their distributional area have they passed much beyond a subtropical environment; and within the tropics, the perennially humid mountain forests in which they have made their homes, shield them from the more severe degrees of heat. In the Himalayan mountains and in Japan they range somewhat above the line at which snow falls annually; but they are most abundant below this line. In Ceylon and most of the remaining countries inhabited by them they are never exposed to snow and ice. The Singhalese species is, however, as I have proved by experiment, capable of enduring a temperature as

low as 7° C. This fact shows that they still retain the hardiness characteristic of Leeches in general.

In Japan the extremes of temperature mark a rather high amplitude; but they are not so far apart as in corresponding latitudes of the neighbouring continent. The surrounding sea and the Black Stream (Kuro-shiwo) are two important factors in determining the climate of Japan; besides giving a milder winter and a cooler summer than are found on the west side of the Japan Sea and the Yellow Sea, they keep the air abundantly supplied with moisture throughout the year. Some idea of the mildness of the winter at Tokio (35½° N.), which lies nearly in the latitude of the localities from which Land Leeches have been obtained, may be gathered from the fact that chrysanthemums appear in October, camellias in December, plum-blossoms in February, and cherry-blossoms early in April. At Tokio the extremes of temperature seldom exceed $-35°$ C. and $-7°$ C. In the thickly wooded, elevated districts inhabited by Land Leeches, the winter temperature will often fall below $+7°$C., and the summer temperature will fall far below the temperature at the same season in Tokio. During the summer, the Japanese Land Leeches enjoy a moderately cool, moist, and very even temperature; in winter they are often covered with snow, and undoubtedly undergo a winter sleep, as in some parts of the Himalayas.

Their capacity for enduring a temperature considerably below the freezing point, their ability to live under water for at least several weeks, and their restriction to perennially moist climates, all show that they have not departed very far, physiologically, from their aquatic predecessors. The untold ages required to scatter them in so many distant and isolated parts of the earth have sufficed to fix them in terrestrial habits of life; but this life has been offered to them under such easy conditions that they have been able to adopt it without fully surrendering their qualifications for the original mode of life.

According to this view, the Land Leeches are not yet fully emancipated from the conditions of aquatic life, since they

can live on land only where the air is loaded with water. They are not, therefore, to be regarded as the scattered and isolated survivors of a race that has passed the meridian of its career, aed are now verging to extinction, but as animals still on the road to terrestrial life.

Although the distribution of these Leeches is now preponderantly insular, there are unmistakeable indications—at least in the case of the Japanese and Singhalese species—that they have sprung from a continental stock. The close affinities between two species so widely separated as those of Japan and Ceylon are easily accounted for, when we remember the proximity of these islands to the same great continent. There can be but little doubt that they are to be explained on the same general principles that serve to account for numerous other resemblances between the faunæ and floræ of these distant islands. I believe that the progenitors of these two species, and probably all the remaining species, had their headquarters somewhere on the continent of Asia, most likely on the slopes of the Himalayas.

HIRUDO NIPPONIA,[1] nov. sp. Pl. XVIII, figs. 10—20.

Diagnostic Characters.

Body has the shape and proportions of the European Medicinal Leech, but is much smaller. Figs. 18 and 20 represent two of the larger individuals, and figs. 12, 14, and 17, three of the smaller ones. The following measurements were taken from one of the larger specimens:—

Length, swimming,	8·5 cm.;	creeping, 10 cm.;	at rest,	3·4 cm.
Width	,,	10 mm.;	,,	7 mm.
Height	,,	3-4 mm.;	,,	4 mm.

Greatest width a little behind the middle; tapering from this point towards the extremities, but more anteriorly than posteriorly.

Cephalic lobe rather broad, and well rounded in front. composed of four annuli.

[1] Nippon, the native name for Japan.

Acetabulum 6 mm. in diameter, circular, and centrally attached.

Annuli 102.—The 5th and 6th annuli coalesce on the ventral side; and the same is true of the 7th and 8th. Counting on the ventral side, and omitting all that are not seen from this side, we find only ninety-three annuli. The first would be the 5th and 6th of the dorsal side; and the second, the 7th and 8th. Behind the 93rd, which is the last that can be seen from the ventral side, there are three more to be seen on the dorsal side, the last of which is very imperfectly defined.

Most of the annuli appear double, when the leech is at rest. To ascertain the whole number of annuli, it is necessary to count from the dorsal side, and to begin with the ring bearing the first pair of eyes. There are sometimes one or more faint indications of rings in front of this point, but they cannot be safely counted.

Buccal Annuli—the 5th and the 6th, the ventral halves of which are united.

Post-buccal Annuli—the 7th and the 8th, also united below.

Genital Apertures.—The male orifice lies in the posterior edge of the 30th annulus (24th counting from the buccals on the ventral side), often appearing in hardened specimens, to lie between the 30th and the 31st. The vulva lies five rings behind the male orifice, in the posterior edge of the 35th annulus, in hardened specimens apparently between this and the 36th. In specimens obtained from Aomori, both orifices were exactly between the above-named annuli.

The male orifice is located between the 2nd and 3rd annuli of the 10th somite; the female orifice, between corresponding annuli of the 11th somite.

Clitellum embraces the 9th, 10th, and 11th somites.

Anus in the 102nd, or last annulus.

Ocelli, five pairs. The first three pairs form a semicircle on the first three annuli, each annulus bearing a single pair of eyes; the fourth pair is placed on the 5th annulus, or first

buccal; the fifth pair on the 8th annulus, or second post-buccal (fig. 10).

Œsophagus has six folds.

Maxilla three, armed with from sixty to seventy straight, conical denticles.

Nephridia, seventeen pairs. The first pair is located in the 6th somite, the seventeenth pair in the 22nd somite. The nephridial pores are placed on the ventral side in the posterior edge of the last annulus of each somite. These pores then occur in the following rings: the 13th, 18th, 23rd, 28th, 33rd, 38th, 43rd, 48th, 53rd, 58th, 63rd, 68th, 73rd, 78th, 83rd, 88th, and 93rd. There are thus four pairs of nephridia before the male orifice.

The vesicles are oval sacs, measuring in sections of hardened specimens 0·8 mm. by 0·6 mm., and bridging only two rings. The three pairs of vesicles situated in the clitellum do not appear to differ in any respect from the rest.

Segmental Papillæ. — Beginning with the 5th ring, which bears the fourth pair of eyes, we find twenty-two papillate rings, in the following order,—5th, 8th, 11th, 14th, 19th, 24th, 29th, 34th, 39th, 44th, 49th, 54th, 59th, 64th, 69th, 74th, 79th, 84th, 89th, 94th, 97th, and 99th. Traces of these papillæ are seen also on the 101st. The dorsal side of each of these annuli, if we except the 5th and the 101st, bears six minute papillæ, a median pair and two lateral pairs. Possibly there may be a marginal papilla on each side, in addition to these, but none was recognised. As these papillæ are regularly placed on every fifth ring, except near the ends, where the intervals are reduced, they may be said to form six longitudinal rows (Pl. XVIII, fig. 10). These papillæ are quite conspicuous in fig. 18, as in this exceptionally coloured specimen each is encircled by a ring of dark brown—a little darker than the pigment of the brown stripes. The area or spot thus encircled is dusky yellow, and shows at the centre a minute round dot that is entirely free from pigment. The papillæ are minute and project only slightly, and the circular areas which they occupy appear as mere pigment spots to the naked eye.

Anteriorly as well as posteriorly these pigment circles become obscure. They are just distinguishable on the 5th and 8th rings, a little more distinct on the 11th, and well defined from the 14th to the 94th. They are small on the 97th, faintly marked on the 99th, and reduced to the merest rudiments on the 101st.

The median spots are arranged along each side the median yellow stripe, projecting somewhat into it and thus causing it to appear contracted or narrowed at regular intervals. The lateral spots are placed along the middle of the narrow dark brown stripes on either side (figs. 10, 18, and 21). The inner rows of lateral spots are directly in line with the eyes, and hence the most anterior of these spots are found on the 11th ring.

Six rows of segmental papillæ occur also on the ventral side, and these are arranged as seen in fig. 13. Here we find two median rows, two lateral, and two marginal. The marginal rows are in the marginal yellow stripe, very near the edge; the lateral rows are a little farther removed from the median ventral line than the nephridial pores, and are about equidistant from the median and marginal rows. These papillæ are considerably smaller than those of the dorsal side, and on this account were for some time entirely overlooked.

Colour.—This species exhibits great variability in colour and markings—so great that when the extremes are placed before us we find it easy to distinguish at least twenty or thirty different patterns. A careful study of these forms has led me to the conclusion that they all belong to the same species, and that their differences are purely individual, and not such as to authorise even the distinction of "varieties." All the figures seen in Pl. XVIII, except 15 and 16, which represent individuals from Aomori, were drawn from living specimens obtained from streams and ponds in and around Tokio. Fig. 19 represents the more common colour and marking, and may be regarded as a typical example of the species; while figs. 14 and 18 show two very wide departures in respect to colour.

The ground colour of the more typical specimens is brownish olive above and pale or yellowish olive below. The typical markings of the dorsal surface are five longitudinal yellow stripes, bordered on each side with very dark brown or black, and usually interrupted (figs. 17 and 19) or blurred (fig. 11) on the first or papillate ring of each somite. The median stripe, which is the broadest and brightest, widens a little on the cephalic lobe between the eyes, and usually terminates behind in a more or less semicircular patch on the acetabulum. The only markings below are two irregular, often nearly obsolete, dark brown streaks bordering the yellow margins (figs. 13 and 20).

The figures of Pl. XVIII have been selected with a view to showing both the degree and the method of variation in colour-markings. The differences in this respect between figs. 18 and 19 are so extreme that it seems at first sight difficult to reconcile them with the fact that the figures represent specifically identical individuals. The specimen represented in fig. 18 was examined closely and found to agree in every particular, except colour, with the common Medicinal Leech of Japan. It was found in a stream that flows alongside the shallow lake known in Tokio as Shinobazu no Ike, where the common Leech is extremely abundant. Among hundreds of Leeches collected at many different times from the same locality this was a solitary example in colour, and hence must be regarded as an individual colour-variety.

An interesting question now arises. Are these colour-varieties mere variations or modifications of what I have described as typical? or are they so many different patterns having no sort of relationship with one another? A closer inspection of the figures shows that the first of these questions must be answered in the affirmative. In fig. 18 the olive shades have almost wholly disappeared, leaving the ground-colour a dull dingy yellow, marked by six irregular dark stripes. If the yellow ground between these stripes be regarded as corresponding to the yellow stripes of most specimens, as plainly indicated by the position of the segmental

papillæ, then we may say that the dark brown stripes correspond to the dark borders of the yellow stripes. But in the typical specimen there are five yellow stripes and twice as many dark borders; how then can these ten borders be represented by six dark stripes? Fig. 10 is an enlarged pencil sketch showing accurately the distribution of the dark pigment of fig. 18. From this figure it will be seen that each of the dark stripes appears to be composed of two parallel halves that have imperfectly blended, leaving here and there evidences of their duplicity. This is especially manifest in the two broader median stripes, and but little less so in the external lateral stripes. These six stripes may then be said to represent twelve dark borders, of which ten ordinarily accompany the five yellow stripes, and two form the inner borders of the yellow margins.

Now, there are three ways in which the dark borders could be made to unite in pairs. First, the widening of the yellow stripes would bring together the six pairs of adjacent borders; second, the widening of the borders themselves, allowing that the yellow stripes persist, would accomplish the same result; third, the obliteration of the yellow stripes would bring together the two borders of each stripe. These three cases are all more or less perfectly represented in the figures of this plate.

In fig. 18 it is the widening of the yellow stripes and the yellow margins that accounts for the arrangement of the dark pigment. That the dusky yellow area enclosed between the two median dark stripes corresponds to the median yellow stripe of the typically coloured specimen, is made sufficiently evident both by its position and by the manner in which it terminates on the cephalic lobe (fig. 18), and on the acetabulum (fig. 10). If this correspondence be conceded, a parallel correspondence must also be claimed for the two lateral dusky yellow areas of each side.

In fig. 11, which represents a portion of fig. 12 magnified four diameters, we have an illustration of the second case, in which there are six dark brown stripes formed, not by the

widening of the yellow stripes, but by replacing the olive-ground colour between these stripes with a brownish black almost as dark as the dusky borders of the stripes. This darkening of the ground-colour is equivalent to widening the six pairs of adjacent dark borders until each pair blends into a single dark stripe. The blending is not quite complete throughout, so that there still remains unmistakable evidence of the double origin of the dark stripes, especially in the inner of the two lateral ones of each side. It will be noticed by comparing figs. 11 and 18 that the segmental papillæ hold the same position relative to the stripes in both cases. A similar case of darkening the ground-colour is seen in figs. 15 *d*, and 20.

An illustration of the third case, in which the two dark borders of the yellow stripe are brought together by the obliteration of the stripe, may be seen in fig. 16 *d*, which represents a portion of a Leech from Aomori; and again in fig. 17, a specimen from Tokio. In the Aomori specimen the external lateral yellow stripes have been completely effaced, the dark borders of each uniting to form a narrow dark stripe on each side. In the two inner lateral stripes, small remnants of the yellow are still to be seen at intervals. The median stripe is a bright lemon yellow, well preserved throughout, and accompanied by the usual dark borders. Both specimens from Aomori show only mere shadows of the dark stripes bounding the yellow margins on the ventral side (figs. 15 and 16). In the specimen from Tokio, it is the two inner lateral yellow stripes that have been wholly effaced, while the external ones are preserved only at intervals. The median yellow stripe is here interrupted on the papillate rings; it broadens as usual on the cephalic lobe, but does not extend to the acetabulum. Here the dark borders of the median stripe are very distinct.

In fig. 14 is represented a specimen in which the yellow stripes and their borders and even the ground-colour have faded. The stripes are barely indicated, and, contrary to the rule, the dorsal side is lighter than the ventral.

The yellow stripes are rarely evenly continuous as in fig. 15,

being generally constricted on the papillate rings (fig. 11) or entirely interrupted (figs. 17, 19).

Only a few examples of this Leech were found in Yezo (officially called Hokkaido), and these agreed so perfectly with those found about Tokio that I am inclined to believe that this island is indebted to the main island for its scanty stock of Medicinal Leeches. In one specimen obtained in Hakodaté, I noticed that the dark borders of the median stripe broadened conspicuously on the middle rings of each somite, which is a feature not infrequent in the Leeches of Aomori and Tokio.

Habitat.—This Leech is very abundant in the ditches and slow streams in the low plains of Tokio, and especially so in the open sewers of this and other cities of the main island. I have occasionally found it in shallow pools in rice fields.

Habits.—Its habits and mode of life are precisely the same as those of the Medicinal Leech of Europe.

Internal Organization.—The structure and relations of the internal organs are almost identical with those in H. medicinalis. There is the same number of ganglia, testes, nephridia, and cæcal appendages of the alimentary tract, and all hold precisely the same relative positions.

The azygous terminal portions of the reproductive organs open beneath the nerve-cords, between the sixth and seventh and between the seventh and eight pairs of ganglia, counting the sub-œsophageal ganglia as the first. The intromittent organ lies on the right, the vagina on the left, of the nerve-cord. The ovaries (Pl. XXI, fig. 65) are small pyriform sacs of about the same size, and occupying the same position with relation to the nerve-cord and the ganglia as the testes. They lie nearly in the same vertical transverse plane with the vaginal orifice, just in front of the vagina. As in H. medicinalis,[1] the oviduct leading from the right ovary passes under the nerve-cord, uniting with the left oviduct at the level of the anterior end of the vagina. The common oviduct (*od. c.*) (oviductus communis) is somewhat tortuous, and its anterior half is enveloped by a mass of unicellular glands, the

[1] Rolleston, 'Forms of Animal Life,' p. 221.

glandulæ albuminiferæ (*gl. alb.*), first made known by Leuckart.[1] This duct lies loosely on the vagina (*v*) and bends into the posterior end of the latter. The vagina consists of a fusiform saccular portion and a narrow tubular portion leading to the external orifice. The saccular portion has about the length of one somite; but it lies opposite the eighth pair of ganglia, so that one half is in the 11th, the other in the 12th somite. The anterior tubular portion appears to be longer than in H. medicinalis.

Remarks and General Considerations.

Name.—I have found no mention of this Leech anywhere except in a few quasi-scientific books of Japanese origin. The more common native name is Hiru, which has, so far as I can learn, only an accidental resemblance to the Latin Hirudo. According to the best information I could obtain, this name has always been in common use among the Japanese; and it is quite certain that it is not a shortened form of Hirudo, as the latter could only have been introduced in comparatively recent times. The same word also signifies garlic, noon, day-time. A similar name, Hiiru, is applied to the mouth of a silkworm.

According to J. C. Hepburn, the name Suitetsu (from sui, to suck, and ketsu, blood) is also applied to the Leech. Neither the Corean name Kōmōri, nor the Chinese Chitsu gives any clue to the origin of the word Hiru.

A Japanese writer, Tanikawa ('Wakunshiori,' vol. xxv, 1830), attempts to explain the matter, by saying that the Leech lives in the mud, hiji, and is therefore called Hiru.

Use.—This is the only Leech used by the Japanese for medicinal purposes. According to an older author, Terashima ('Wakansansaidsuye,' vol. lii, 1713), the Japanese have not only employed the Leech in the common way, externally, but also as an internal medicine. As an example, the writer says that the Leeches are dried and reduced to fine powder, of which about

[1] 'Die menschlichen Parasiten,' i, p. 679, 1863.

eight grains are taken with saké (rice-wine) to cure "sessho totsu" (which was interpreted to me as pains resulting from broken limbs). If the pain continues, a second dose is taken, which soldom fails to bring relief!

In external use the Leech is applied by the aid of a bamboo tube.[1]

The Diagnostic Value of the Annuli.—In the past descriptions of Leeches, there has been a growing recognition of the fact, that the number, character, and metameric combination of the annuli furnish important marks for the determination and comparison of species. Gratiolet and Grube are the only authors, however, who have shown any very clear appreciation of this point. The general neglect in this respect is doubtless attributable to the difficulty in counting and describing accurately the annuli on the two ends of the body, as well as to a lack of appreciation of their importance for systematic purposes. The result is that, up to the present moment not a single description of any Medicinal Leech has been given with sufficient completeness for a close and full comparison of even its more important external characters with those of other species. More than this, it would be impossible, from the innumerable monographs, memoirs, and stray papers on the Medicinal Leech, to patch up a description that would fully meet the obvious requirements for a critical comparison of any two species. I am well aware of the import of these statements, for my experience has given me a keen sense of their meaning. So far as the matter in hand is concerned, I venture to say that by far the greater number of the species-diagnoses that have been showered upon us from time to time, have been so superficially and slovenly done, that it would puzzle the perpetrators to identify the species they profess to have described. I wish here to insist on the importance of a thorough study of the annuli of the Leech, particularly those of the abbreviated terminal somites, as a means of making clear the precise position and relation of the parts

[1] For these references to Japanese literature, I am indebted to Mr. Tanada, who was my assistant in the zoological laboratory at Tokio.

which assist in the determination of species. I have satisfied myself that not only the number and position of the rings, but the relative size and general appearance of each ring even to very minute details,[1] are accurately reproduced in every normal individual of a species. The obscurity that is supposed to exist in regard to the precise number of rings which enter into the composition of the cephalic lobe or the hind end of the body, affords no excuse for the meagre descriptions usually given of these regions, but furnishes rather an argument for describing them with the utmost care and detail. As to the difficulties in the way of counting, these are scarcely worth mentioning in the various species of Hirudo, or of the allied genera, Aulostoma, Hæmopis, Macrobdella, &c. It is only necessary to adopt some method of counting that can be safely followed in all these genera. What my own method is, I have made clear in the foregoing descriptions; and it now remains only to show its advantages over those proposed by other writers. As before pointed out when comparing the Land Leech with the Medicinal Leech of Japan, it will not do to follow Moquin-Tandon, Diesing, and others in counting from the ventral side, for some of the more important rings are not seen from this side; and the dorsal aspect of some rings, particularly the buccals and post-buccals, differ very much from the ventral. Besides, the abbreviated somites can only be clearly described by an accurate study of the dorsal side; and it is here that the sense-organs attain their highest development, and the colour-markings their more important diagnostic distinctions. The total number of annuli, the position of the sexual orifices, the nephridial pores, and the segmental papillæ, must therefore all be determined by reference to the dorsal side, the differences between this side and the ventral being noted wherever necessary.

A still more objectionable method is that of counting the annuli from the anterior end, but from two different points, one on the dorsal the other on the ventral side. Thus the organs of the two sides, being located with reference to two

[1] Colour alone excepted.

different starting-points, are thrown out of relation, and confusion is the consequence. The confusion consists in this, that the dorsal and ventral halves of the same ring bear two different numbers. In the case of Macrobdella decora, for instance, the dorsal half of the buccal ring, according to Verrill, is counted as the 6th, five rings preceding it; while the ventral half is called the 1st, starting from the mouth. In the same way the male orifice is said to lie in the 27th ring behind the mouth : but what is the number of this ring on the dorsal side ? It is certainly a very simple matter to add five, the number of rings supposed to belong to the cephalic lobe, to twenty-seven; but this alone would not give us the number on the dorsal side in any Leech which, like the Medicinal Leeches of Europe, China, and Japan, has four rings (two buccals and two post-buccals) represented by two on the ventral side. The simplest method, and the one least liable to confusion, seems therefore to be that of numbering the rings from one fixed point on the dorsal side. Each ring then has a definite number and precise relations.

Gratiolet was the first to emphasize the importance of a well-defined starting-point in counting, as a means of determining with precision the position of the genital pores. Under the persuasion that no such point could be found on the dorsal side which would be convenient in use, he recommended the posterior pair of nephridial pores as the most satisfactory point of departure, reckoning from this point forward. The considerations which led him to adopt this unconventional and somewhat awkward method, may be seen from the following:

"La chose importante dans cette recherche serait de partir d'un point fixe et nettement défini. Or, la plus grande incertitude régnant sur le nombre des anneaux aux deux extrémités de l'animal, il faudrait en conséquence pouvoir les négliger. En y réfléchissant un peu, le problème ne paraîtra pas absolument insoluble. Quand on étale une Sangsue morte ou vivante, et qu'on la fait glisser sur sa face dorsale appliquée sur la convexité du doigt indicateur, on aperçoit, d'espace en espace, deux petites gouttelettes de liquide symétriquement accumulées sur le bord postérieur de certaines anneaux. Ces

gouttelettes s'échappent de petits orifices qui conduisent par un canal oblique et fort étroit, à certaines vésicules intérieures, dont nous parlerons dans un instant. Ces orifices, ainsi que nous venons de le dire, sont disposés en paires symétriques, et ces paires sont séparées les unes des autres par des intervalle. qui, à la partie postérieure du corps, comprennent régulières ment cinq anneaux. Or elles sont au nombre de dix-sept, et par conséquent, si le nombre des anneaux compris dans ces intervalles est fixe entre la première et la dernière, il y a nécessairement quatre-vingts anneaux. Malheureusement ce chiffre n'est par exact; en effet, le nombre des anneaux varie à l'extrémité antérieure de la série, où d'ailleurs les orifices sont très difficiles à discerner. Le seul point fixe, ou du moins le plus commode, se trouve dans la paire postérieure d'orifices qui est toujours distinct et facilement apparente . . . .

"Le nombre des anneaux intermédiaires décroît vers l'extrémité antérieure de la série; c'est ainsi que le quinzième intervalle, compté d'arrière en avant, n'a que quatre anneaux, et le seizième trois seulement; dès lors, le nombre total des anneaux, compris entre les deux paires extrêmes d'orifices, n'est pas de quatre vingts anneaux, comme on aurait pu l'admettre à priori, mais de soixante-dix-sept (l. c., p. 10, 11).

The objections to counting from the ring bearing the last pair of nephridial pores are:

1. It is an unnatural and confessedly a forced method.
2. It does not answer all the ends that may be reached by beginning with the first pair of eyes.
3. It is an attempt to evade the difficulties involved in the obscurity of the rings at the two extremities.
4. It is necessarily limited in its application to those few genera in which the posterior pair of nephridial pores are sufficiently distinct to be easily recognised.

The examination of the abbreviated somites has already revealed to us a natural, convenient, and precisely defined starting-point for counting in Hæmadipsa and Hirudo. For reasons before given, it is certain that the first three pairs of eyes in Hirudo mark three successive rings. Beginning

then with the first pair of eyes, we find the fourth and fifth on the fifth and eighth rings respectively. Now this simple arrangement of the eyes which is only slightly modified in Hæmadipsa, holds good not only for Hirudo, but for Hæmopis, Aulostoma, Macrobdella, and all the more closely related genera. From the fifth pair of eyes onward, the counting is rendered more easy by the size of the rings, as well as by the metameric arrangement of the colour-markings and the segmental papillæ. It is certainly very desirable that the various species of the above-named genera should be described on a common plan. It seems to me that for simplicity and clearness there is no better method than the one here recommended. It is quite certain that no clearly marked ring exists anterior to the first pair of eyes that would serve the purpose we have in view. There are here, to be sure, in some species, obscure traces of what, in the opinion of some authors, might be regarded as one or two rings. While it is important to take note of all such evidences of rings, it is certainly advisable, for the sake of uniformity, to discard them in counting.

Abbreviated Somites.—The comparison of Hæmadipsa with Hirudo nipponia has shown that we cannot afford either to ignore the rings composing the two ends of the body, nor to pass them over with such imperfect descriptions as are usually accorded to them. That the terminal somites are more or less abbreviated or shortened, by suppression of rings, is a fact recognised by all recent writers; but no one has hitherto thought it necessary to give more than a very superficial account of them. Gratiolet's method of counting was adopted with a view to avoiding a close study of these somites; and, certainly, it is admirably adapted to this end. Fortunately, the position of the five pairs of eyes has been sufficiently well-defined to enable us to understand the composition of the first four somites in Hirudo and cognate genera; beyond this, our information is too meagre and indirect to settle either the number or the composition of the abbreviated somites.

Gratiolet finds one hundred and two annuli in H. medicinalis, as will be seen from the following figures:

From tip of head to first pair of nephridial pores . . .	10
Between first and last pair of nephridial pores . . .	77
Between last pair of pores and anus	9
Acetabulum	6
Total number	102

Following the same order in the case of H. nipponia omitting the acetabulum, we have:

From tip of head to first pair of pores	13
Between first and last pair of pores	80
Between last pair of pores and anus	8
Between anus and acetabulum	1
Total number	102

The most important difference here is found in the number of rings that separate the two extreme pairs of nephridial pores. In the Japanese Leech (fig. 10), there are sixteen unabridged somites between these two points, the 7th to the 22nd inclusive. In the European Leech, according to the statements cited from Gratiolet, the 7th somite is composed of three rings, and the 8th of four rings; the remaining fourteen containing each five rings. Thus, if we accept Gratiolet's statements, we must allow that H. medicinalis has eight abbreviated somites at the anterior end, while H. nipponia has only six abbreviated at this end. Now such a difference is, as will be shown in the sequel, quite irreconcilable with the opinion that the two species belong to the same genus. In order to remove all doubts as to the propriety of placing the Japanese Leech in the genus Hirudo, I have examined a considerable number of Medicinal Leeches from different parts of Europe and Asia, as well as Aulostoma, Hæmopis, and Macrobdella Verrill. This examination has brought to light some facts concerning the composition of the body of the Leech, which has hitherto escaped notice, facts which will serve as a basis for comparative systematic studies,

and at the same time as a most important guide to the genealogical relationship of the various species and genera.

The Genus Hirudo.—Every Hirudo has twenty-six somites, counting from the first pair of eyes to the acetabulum: ten of these—the first six and the last four—are abbreviated by the suppression of from two to four rings in each; and sixteen, lying between the first and the last pair of nephridial pores, have each five rings. The six anterior somites include thirteen rings,—the 1st and 2nd being represented each by a single ring, the 3rd by two rings, and the 4th, 5th, and 6th, each by three rings. The four posterior somites embrace nine rings (94—102), the 23rd somite including three rings, and the 24th, 25th, and 26th, each two rings.

The first ring of each somite is marked, anteriorly, by a pair of eyes; and, from the 11th ring onward, by the segmental papillæ, of which there are normally from six to eight on the dorsal half of the ring and six on the ventral half.

The serial homology of the segmental papillæ and the eyes is apparent from their arrangement; for the first pair of eyes replace a pair of median papillæ; and the remaining four pairs of eyes replace as many pairs of the inner lateral papillæ.

The eye-bearing rings are the 1st, 2nd, 3rd, 5th, and 8th.

The buccals are the 5th and 6th, which are united on the ventral side. The post-buccals are the 7th and 8th, also united ventrally.

The first pair of nephridial pores is situated in the 13th ring; and the last (17th) pair in the 93rd ring.

The male orifice lies between the 30th and the 31st ring, the second and third of the 10th somite. The female orifice is five rings behind the male, and

thus holds a similar position in the 11th somite, between the 38th and the 36th ring.

The anus lies in the 102nd ring, or between this and the preceding one.

The other characters of this genus, such as the maxillæ, denticles, alimentary tract, reproductive organs, nephridia, &c., are too well known to require repetition here.

I shall presently bring abundant evidence to show that the above characters are typical of Hirudo; and that any well-marked departure from this type, in the total number of somites, in the number or composition of the abbreviated somites, with perhaps the exception of the 26th somite, in the number or position of the eyes, nephridial pores, sexual orifices, &c., cannot be consistently admitted for any species included in this genus. If this conclusion be correct, some names will certainly have to be expunged from our lists of genera; but no objection on this ground can outweigh the advantages of a clearly defined and convenient standard of comparison. When we remember that naturalists began by referring almost every Leech, even Clepsine, to the genus Hirudo; and that some of our more recent authorities have gone to the other extreme, of setting up new genera on distinctions of doubtful significance, it becomes evident that a genus should stand for something more than a name coupled with a few observations that leave us in the lurch whenever we seek to know its precise limits and relations to other genera.

A close comparison of the Japanese Medicinal Leech with those imported from Sweden, and with those which I collected myself in Saigon, Singapore, Ceylon, and Naples (Sebeto River), enables me to say that all the characters above named are common to these widely separated and distinctly marked species. It is more than probable, therefore, that Gratiolet was in error as to the number of rings in the 7th and 8th somites.

Aulostoma and Hæmopis.—I have been surprised to find such a close agreement among the different species of Hirudo, in regard to the number and character of the rings as

well as the number and composition of the abbreviated somites; and still more so, to find these characters repeated with all the more important details of number and position in both Hæmopis, and Aulostoma. This certainly indicates a close relationship between the three genera. Aulostoma is, however, a well-founded genus, distinguished from Hirudo by its habits, mode of life, form of its alimentary canal, character of its teeth, and the position of the male orifice, which is in the middle of the 31st ring, instead of between this and the 30th. In the case of Hæmopis, the distinctions are so few and unimportant that it is difficult, if not impossible, to justify a separation from Hirudo. Hæmopis is a complete copy of Hirudo in all the particulars before named, and its highest claim to generic rank is based on the small number of its denticles. In view of the great variability in the number of the denticles, not only among different species of one and the same genus but also among individuals of the same species, and even in the different jaws of the same individual, this distinction hardly deserves generic rank. The other distinctions on which this genus rests, whether considered singly or collectively, are even less satisfactory as generic characters. Leuckart[1] long ago declined to recognise Hæmopis as a distinct genus. After defining the genus Hirudo, he remarks:—"Thus characterised, the genus Hirudo embraces not only the larger number of species hitherto referred to it—with the exception, e.g. of H. lateralis, Say—but also the genus Hæmopis, the separation of which we must regard as unsound so long as the usual distinctions ('body less flat, less deeply annulated at the margin, in contraction less olive shaped, denticles less numerous') are not replaced by others of a more positive value."

Hirudo and Hæmopis both require the same food, and obtain it from the same sources and by the same means, with the single difference, that Hæmopis, which is provided with denticles too short and dull to make an incision in the epidermis, is restricted in its attacks to epithelial surfaces which

[1] Leuckart, 'Die menschlichen Parasiten,' i, p. 716, 1863.

are easily sawn asunder, such as are found in the mouth and the nostrils.

The genus Hæmopis appears thus to rest on an insufficient basis; and, as its rejection will be more consistent with our present nomenclature than its retention, I venture to propose its reabsorption in the genus Hirudo.

Does then the number of denticles furnish any guide in the determination of genera? Is there any point in the reduction of the number of denticles which can be taken as a limit to the genus Hirudo? All will agree that there is at least one such point; and I think a little reflection will show that there is only one. So long as the denticles are sufficiently numerous and well formed to enable the Leech to live by sucking blood, it is plain that the reduction has not reached a point at which the formation of a new genus becomes imperative. When, however, the number and efficiency of the denticles have been reduced to such an extent that the Leech becomes incapable of drawing blood, and is thus compelled to accept a different kind of food and to adopt new methods of obtaining it, it is obvious that the boundary line between two very distinct courses of life has been passed. The degeneration of the denticles has been carried to a point that necessitates a complete revolution in habits, and a whole train of correlated morphological changes sweep in. Such has been the history of Aulostoma. The climacteric limit in the reduction of the number of denticles lies between Aulostoma and Hæmopis, and this limit is the only one which, in this direction, can be found for the genus Hirudo. Between the maximum and minimum number of denticles compatible with the life of Hirudo, I can see no limit to variation that is entitled to generic rank. Any attempt to establish a limit to the genus inside of these extremes, must be pronounced irrational, since it makes it impossible to draw any line between specific and generic distinctions. The futility as well as the absurdity of such an attempt has been shown in the use that has been made of the genus Hæmopis. Various Land Leeches have been referred to Hæmopis, not on account of any real generic affinity, but simply

because they were said to have fewer teeth than are usually found in Hirudo medicinalis. It is evident too that certain aquatic Leeches, although much further removed from Hæmopis than this genus is from Hirudo, have, nevertheless, been associated with the former on the same insufficient ground. A similar blunder has been made in the attempt to make the entire absence of denticles a basis of generic association. The discovery of toothless Leeches in different parts of the earth, which have evidently descended from different species of denticulated Leeches, shows how unreliable and worthless genera are when founded on such characters. But if the entire absence of denticles is no certain indication of generic affinity, how much less certain is a difference in number only. I have satisfied myself that two Leeches belonging to two distinct genera may often agree more nearly in the number of denticles than two species of the same genus. Numerous instances of this kind are at hand, but one or two will be sufficient here. No one will deny that Macrobdella, Verrill, and Hirudo are quite distinct genera. Now Leidy[1] has described a species of Macrobdella with fifty-five teeth; and Schmarda[2] states that Hirudo quinquestriata (from Australia) has from forty-eight to fifty teeth. It is also stated by Schmarda that H. multistriata (Ceylon) has about one hundred teeth.

Again, Macrobdella floridana Verrill, has only "about twenty acute teeth,"[3] thirty less than Macrobdella, Leidy. In M. sestertia (n. sp.) I have found one jaw furnished with thirty-nine, the second with forty-three, and the third with forty-six teeth. Schmarda found only thirty teeth in Hæmopis ceylonica, which is a Land Leech belonging to Hæmadipsa—a genus sufficiently distinct from Macrobdella.

If we are to avoid increasing the number of genera until they equal or nearly equal the number of species, it is evident

[1] Leidy, 'Proc. Phil. Acad. Nat. Sci.,' p. 230, 1868.

[2] Schmarda, 'Neue wirbellose Thiere,' i, 2nd part, p. 2, 1861.

[3] Verrill, 'Synopsis of the North American Fresh-water Leeches," p. 669, 1874.

that we must find some better basis for distinguishing genera than has yet been offered in the case of Hæmopis.

The Somites as a basis of Classification.

I shall conclude my remarks on the somites as a basis for distinguishing genera, by a comparison of genera from different countries. That such an important basis of classification has thus far been completely ignored, is due to the fact that the segmental papillæ have hitherto attracted very little attention. It is the metameric arrangement of these peculiar sense-organs, which I regard as incipient eyes, which has revealed to me the degree of abbreviation that has taken place in the terminal somites, and thus led to the discovery of characters which serve to fix precise limits to genera, and to determine their phylogenetic relationship. The following descriptions, added to those already given of Hæmadipsa and Hirudo nipponia, will make clear the facts on which some of the foregoing conclusions rest.

1. Hirudo medicinalis (Sweden). In order to define the position of the segmental papillæ, the colour markings must be briefly noticed. In the specimen examined the dorsal surface was marked by six brownish-yellow stripes. The two median stripes were about one third of the width of the body distant from each other, and thus divided the dorsal surface into a median and two lateral areas. The lateral stripe of each side lay near the median stripe, separated from it by considerably less than half the width of the lateral area. On every fifth ring (last of each somite) the lateral stripe inclosed a more or less triangular black spot, the more elongated angle of which pointed forward. Similar spots, but much smaller, were also seen in the median stripes on the same rings. The two latero-marginal stripes were very narrow and were separated from the yellow margins by a narrow black stripe. This black stripe widened on the last ring of each somite, in the direction of the middle dorsal line, thus causing the latero-marginal stripe to form a curve at these points in the same direction. The median and lateral stripes coalesced on each side near the

hind end of the body, and were then continued as one stripe to the edge of the acetabulum. The same stripes coalesced also anteriorly.

The ground-colour of the dorsal surface was a dull olivaceous green ; the ventral sarface was pea green thickly flecked with black. The yellow margins were bounded on the ventral side by a broad black stripe, the inner edge of which was quite uneven.

The dorsal half of the papillate rings bears eight segmental papillæ ; the ventral half six. On the dorsal side (Pl. XX, figs. 47, 49) we see two median rows of papillæ (*m.*), located on the following annuli—2, 3, 5, 8, 11, 14, 19, 24, 29, 34, 39, 44, 49, 54, 59, 64, 69, 74, 79, 84, 89, 94, 97, 99, 101 ; two inner lateral rows (*il.*), beginning on the eleventh annulus, and following the line of the lateral stripes to the posterior end of the body ; two outer lateral rows (*ol.*), near the outer edge of the lateral areas of colour, beginning as far forward as the fifth annulus and traceable to the 101st annulus ; and two marginal rows (*mg.*), located in the narrow latero-marginal yellow stripe. On the ventral side (fig. 48) we find two marginal rows (*mg.*), located in the inner edge of the yellow margins of the body ; two lateral rows (*l.*), just inside the black stripes ; and two median rows (*m.*), separated from each other by a little less than one third the width of the body.

The median rows of the dorsal side exhibit serial relationship with the first pair of eyes ; while the inner lateral rows, which are larger and more conspicuous than the others, show a similar relationship with the remaining pairs of eyes.

Counting from the first pair of eyes to the anus, there are twenty-six somites, of which six at the anterior and four at the posterior end are abbreviated, leaving sixteen full somites between the first and last pair of nephridial pores. The total number of annuli is 101, of which thirteen belong to the first six somites, eighty to the sixteen full somites, and eight to the last four somites. The 1st and 2nd somites are each represented by a single ring ; the 3rd by two rings ; the

4th, 5th, and 6th, each by three rings; the 23rd somite includes three rings; the 24th and 25th each two rings; and the 26th one ring, with an occasional rudiment of a second (the 102nd).

The 5th and 6th rings, designated as the buccal rings, completely coalesce on the ventral side; and the same is true of the two post-buccals (7th and 8th). This apparent coalescence really means the suppression of the ventral half of the non-papillate ring.

The nephridial pores are placed in the posterior edge of the last ring of the somite; the first pair (1st p.) in the 13th ring, and the seventeenth pair in the 93rd ring.

The male orifice lies two rings behind the fourth pair of pores, between the 30th and the 31st ring; the female orifice lies five rings behind the male, between the 35th and the 36th ring.

The anus lies behind the 101st ring. The post-anal ring (102nd) is at best only rudimentary, and can only doubtfully be claimed as belonging to the body.

In the Medicinal Leech found in Sebeto River (Naples), we have only a few differences to note. The two buccals are faintly demarcated on the ventral side, while the post-buccals are fully united. The total number of rings is 102, and the anus lies in the 102nd ring, nearly dividing it (fig. 50).

Except in colour-marking and size, the European Hirudo agrees almost perfectly with H. nipponia.

2. Aulostoma of Leipsic and Naples. Whether the Aulostoma of Leipsic is specifically distinct from that of Naples or not, is a question that I am not prepared to answer decisively; and I will not therefore prejudge the case by giving them different names. My examinations have been made mainly for the purpose of ascertaining how far the somites correspond to those of Hirudo.

The total number of rings between the first pair of eyes and the anus is 100, one less than in Hirudo; but this difference turns out to be of subordinate importance. As shown in fig. 52, the anal aperture is very large, completely dividing two

narrow rudimentary rings (101 and 102), and even encroaching somewhat upon a third (100). This figure represents a specimen obtained at Leipsic. In specimens from Naples, the last two rings (99 and 100) show signs of duplicity at their margins; and in one or two cases, it is perfectly evident that both of these rings are double, the 99th corresponding to the 99th and 100th in Hirudo, and the 100th to the 101st and 102nd. Allowing that such a correspondence actually exists, we should expect to find segmental papillæ on both of these rings. They are always present on the 99th ring, and in a few cases they are quite distinct on the 100th ring. It is safe to conclude therefore that Aulostoma has the same number of rings as Hirudo, with the difference that in the former the 100th and the 102nd, which are non-papillates, are less distinct than in the latter. In the Leipsic specimens (fig. 52) the 101st and 102nd rings are very distinctly indicated, but not in the same way. It is not improbable that the difference here pointed out may have the value of a specific distinction.

The 23rd somite has three rings, but it is noticeable that two of these rings (95th and 96th) are much thicker than the preceding or the following rings, showing that the abbreviation of this somite has not been carried quite so far in Aulostoma as in Hirudo. The abbreviation of the 25th and 26th somites, on the contrary, is somewhat more extreme than in Hirudo. With respect to the anterior abbreviated somites, I am unable to point out any differences between the two genera.

The nephridial pores hold the same positions and relations in both cases, and there is only a difference of half a ring in the position of the male orifice, which generally occupies the middle of the 31st ring,[1] instead of lying between the 30th and the 31st.

The number and arrangement of the segmental papillæ are the same as in Hirudo; but the median dorsal and all the ventral papillæ are less strongly developed. In some specimens the papillæ are quite distinct on the acetabulum (fig. 52).

The denticles are from eleven to fifteen in number, but vary

[1] I have sometimes found this orifice in the anterior edge of the 31st ring, very near the line dividing this from the preceding ring.

considerably in different individuals in respect to the degree of development attained.

3. Hirudinaria javanica (Hirudo javanica Wahlberg).—Dr. C. Ph. Sluiter, of Batavia, has been kind enough to send me some very excellently preserved specimens of this interesting Leech, together with drawings and full descriptions of the colour-markings. I have given the alcoholic specimens a thorough examination, and am able to add some facts to those communicated by Dr. Sluiter.

This Leech resembles, in some respects, Hirudo maculosa, Grube,[1] but differs from it and from all other Medicinal Leeches known to me in two very striking peculiarities, namely, the separation of the sexual pores by seven instead of five rings, and the enormous size of its acetabulum, which reaches forward to the level of the last pair of nephridial pores. The first-named distinction alone appears to me quite sufficient to justify its separation from Hirudo, and it is on this ground that I propose to give it the generic name Hirudinaria.

This genus agrees with Hirudo in the number and composition of its somites, and in having precisely 101 rings between the first pair of eyes and the anus. The dividing line between the buccal rings (5th and 6th) extends to the ventral side, but vanishes before reaching the median line of this side. The post-buccal rings are somewhat more perfectly united on the ventral side. The male orifice lies between the 30th and 31st ring, the female orifice between the 37th and 38th ring. The 102nd ring, which forms a sort of neutral zone between the body and the posterior sucker, is broken into two lateral halves by the anus (fig. 56).

The maxillæ are very large and the denticles unusually numerous (115—130). The inner angle of the maxilla (fig. 60, *i*) rises abruptly above the level of the œsophageal fold which it terminates, and the lateral surfaces exhibit a consid-

[1] Ed. Grube, "Anneliden," in 'Reise der Oesterreichischen Fregatte Novara um die Erde in den Jahren 1857—1859,' Zoologie, Abth. 3, B. ii, Wien, 1868, pp. 39—40.

erable number of wart-like protuberances. The denticles are straight, conical, and radially directed; the longest (·035 mm.) are placed at the inner angle of the maxilla, from which point they diminish gradually in length towards the external angle, where they vanish in the merest rudiments.

In a specimen measuring 85 mm. in length, the œsophagus measured 6 mm. (excluding the maxillæ) ; the anterior half shows only three folds, one median dorsal and two latero-ventral. Each of these folds divides into two near the middle of the œsophagus, thus making six folds in the posterior half.

The segmental papillæ are remarkably large, resembling in form, size, and inclination those seen in the Medicinal Leeches of Saigon, Singapore, and Ceylon. In number and position they agree with those of Hirudo. In no other species have I seen such an extraordinary number of these papillæ on the acetabulum. Their arrangement here shows that the acetabulum is composed of at least eight somites, each of which is now represented by a single papillate ring. The abbreviation is thus carried further here than in any other portion of the Leech except in the cephalic lobe.

The median papillæ (*m.*) incline a little towards the median line of the dorsal surface ; the inner lateral papillæ incline still more in this direction; and the outer lateral (*ol.*) and the marginal papillæ (*mg.*) have their longer axis directed nearly at right angles to the axis of the body.

I am wholly indebted to Dr. Sluiter for the following description of the colour and markings of this species, which was very imperfectly described by Wahlberg :[1]

"Ground-colour of the dorsal side dull olive green, sometimes inclining more to grass green, at other times more to brownish shades. In the median line of this side there is a series of elongated black spots, from twenty to twenty-five in number, which never blend into a continuous stripe. Towards the head these spots are smaller and often more rounded, while in the middle and posterior region they are more elon-

[1] Öfvers, 'Kongl. Vet.-Akad. Förh.,' Stockholm, 1855, p. 233. Compare Diesing's 'Revision der Myzhelminthen,' Abth. Bdellideen, p. 38, Wien, 1859.

gated, stretching over three annuli. This series of black spots lies in a broad stripe of a lighter colour than the ground-colour, which is narrowed at each of the intervals left between the black spots. On each side of this broad stripe are two narrow, longitudinal yellowish stripes, each of which is bounded by two narrow black borders. These lateral stripes are interrupted from point to point, so that they do not form unbroken stripes. The entire dorsal surface is flecked with black, and these flecks are more numerous and larger along the yellow margins. The dorsal side of the margin is a clear yellow, while the ventral side is reddish yellow. Often the yellow margins are very regularly dotted with black, a single dot occurring on each ring. A few irregular larger black flecks are also seen scattered along these margins.

"The ground-colour of the ventral side is brick red; just inside the yellow margins of this side are two broad stripes of the same dull green as the ground-colour of the dorsal surface; these stripes are sharply defined against the brick red middle zone by an intermixture of black flecks, which for the most part blend together. The two suctorial surfaces are bluish grey, the oral surface being a little lighter than the posterior sucker. The oral surface has a pale margin, which is not seen in the acetabulum.

"The eyes are placed on the 1st, 2nd, 3rd, 5th, and 8th rings, as in H. medicinalis.

"Length = 175 mm. . . . .

"I found some Leeches which agreed in general with the above description, but which showed a constant difference in colour, and which are probably to be regarded as a variety. The dorsal surface was less variegated in colour, without the lateral stripes, and darker green. The black flecks and stripes were the same. The ventral surface is not brick red, but of the same green colour as the dorsal side, without the black flecks. The dark stripes inside the yellow margins are broader and have a larger admixture of black. Large and small individuals of both varieties were found, from which we may conclude that the difference is not one of age. Both varieties

are very abundant in the Sawahs (rice-fields), in the water of the low lands around Batavia and elsewhere on the north coast of Java. The Malayan name is Lintah. Both varieties are used for medicinal purposes."

4. Leptostoma.—Three species of Japanese Leeches (Pl. XVIII, XIX, and XX) agree with the forms hitherto mentioned in having twenty-six somites between the first pair of eyes and the acetabulum, but differ from all of them in having fewer abbreviated somites. This peculiarity shows that these Leeches have not descended from Hirudo medicinalis, and that they are entitled to rank as a more primitive type than any of the Hirudinidæ at present known. These Leeches possess certain characters (denticles rudimentary or absent) that suggest relationship with Aulostoma; but Aulostoma is unquestionably an offshoot from Hirudo, and the characters in which it approaches Leptostoma cannot be regarded as evidence of genetic affinity. The rudimentary condition of the denticles and maxillæ, with all the correlated peculiarities, are characters that have been acquired independently by the two genera. Leptostoma and Hirudo, we must assume, had a common ancestral form; and Leptostoma has departed from this archaic form in much the same way that Aulostoma has departed from Hirudo. This seems to me to be the most rational mode of explaining the relationship of these genera.

Pl. XX, figs. 54 and 55, will show the more important characters on which the new genus Leptostoma is based. These figures represent the two extremities of Leptostoma pigrum somewhat diagrammatically. Looking first at the anterior end (fig. 54), we find only five abbreviated somites. These five somites contain the same number of annuli (10) as the corresponding somites in Hirudo; but there is a small difference to be noted in the last ring of the 5th somite. This ring is constantly larger than the other rings, and hence it may be regarded as representing two rings combined. The 6th somite includes five annuli, two more than the same somite in Hirudo. This difference explains other differences; for instance, the position of the first pair of nephridial pores

in the hind edge of the 15th annulus instead of the 13th, and the location of the genital pores between the 32nd and 33rd, and between the 37th and 38th rings. Passing to the posterior end of the body (fig. 55), we find here only three abbreviated somites, the 23rd somite containing the full number of rings. Thus there are eighteen unabbreviated somites (sixteen in Hirudo) and eight abbreviated (ten in Hirudo). It is interesting to note that the abbreviated somites have been abbreviated to very nearly the same extent as in Hirudo. As we have here two more complete somites than in Hirudo, we have 106 annuli between the first pair of eyes and the anus. The nephridial pores occupy the same somites (six to twenty-two inclusive) as in Hirudo, and hold homologous positions; for the 15th and 95th annuli are here homologous with the 13th and the 93rd in Hirudo. The 106th annulus is homologous with the 102nd of Hirudo. The 103rd annulus (99th of Hirudo) is plainly double at its margin, though single elsewhere; and the 102nd annulus is constantly thicker than the preceding ring, which indicates that it represents two rings consolidated. There is abundant evidence that the somites are not abbreviated by a sudden and complete syncopation of one or more annuli; the process is rather a gradual one, consisting in the coalescence of two successive annuli. When a papillate annulus combines with a non-papillate, as seen in the 103rd, the individuality of the latter seems to be suppressed, in subordination to that of the former. In this case (103rd annulus) it is evident that the posterior half of the annulus represents the original papillate annulus, as shown by the position of the papillæ, and thus it becomes plain that two successive annuli of different somites may combine.

Leptostoma edentulum (Pl. XIX) agrees very closely with L. pigrum having 105 rings, and sometimes a fragment of a 106th. The number and abbreviation of the somites are essentially the same. Only one difference requires mention here: in the 23rd somite, the 97th and 98th annuli are often not so plainly divided as the following rings of the same somite. This peculiarity is not apparent in L. pigrum, while

it has been carried one step further in L. acranulatum (fig. 53). Here the 97th annulus represents the 97th and 98th (of L. pigrum and L. edentulum) fully consolidated as may be inferred from its size. We have thus only 104 annuli, with sometimes a trace of a 105th. These three species agree in having five abbreviated somites (embracing ten annuli) at the anterior end, and in the position of the genital orifices and nephridial pores. Their chief point of difference is the degree of abbreviation represented in the 97th and 98th annuli.

5. Macrobdella sestertia,[1] nov. sp.—As the Macrobdella which I have examined differs in some important points from those described by Verrill, Leidy, and Brooks, I shall give a full description of the specific as well as the generic characters.

Diagnostic Characters.

Body has the shape and proportions of Hirudo of Europe, except that, anteriorly, it tapers rather more rapidly. The following measurements were taken from a middle-sized specimen: Length, swimming, 9·5 cm.; in extension, 13 cm.; at rest, 5·8 cm. Width, swimming, 12 mm.

Cephalic lobe, semi-ovate, smaller proportionally, than in Hirudo; composed of four annuli. The thin margin is capable of considerable extension and is slightly emarginated at the tip; it is thickly beset with fine papillæ on its inferior surface. The under side of the cephalic lobe shows three convergent fossæ, one median corresponding to the dorsal maxilla, and two lateral corresponding to the latero-ventral maxillæ. When the Leech is at rest the head is usually rolled into the buccal cavity, as is the habit with all Hirudinidæ.

Acetabulum circular and centrally attached; 6·5 mm. in diameter.

Annuli 103; the last three very imperfectly marked. Most of the annuli appear to be double; but the two halves are separated by a comparatively shallow furrow.

[1] This name is given in allusion to the fact that the sexual openings are separated by two and a half rings.

Buccal Annuli = 5th and 6th; distinct on the ventral side, but not so deeply divided as the following annuli.

Post-buccal annuli = 7th and 8th, distinct below.

Genital Apertures.—The male orifice is in the middle of the 32nd annulus (4th annulus of 10th somite); the female orifice lies between the 34th and 35th (1st and 2nd of 11th somite), separated from the male by two and a half annuli.

Clitellum embraces 9th, 10th, and 11th somites.

Copulatory Glands. ("mucous glands," Brooks).[1]—A quadrangular swollen area occupies nearly the median third of the 41st, 42nd, and 43rd annuli, on the ventral side. This area is divided into an anterior and a posterior half by a groove running along the middle line of the 42nd annulus, so that each half occupies the width of one annulus and a half (fig. 57, Pl. XX). In each half is a row of six small oval areas, pale or flesh coloured, side by side; and in each of these two gland pores. Thus there are twenty-four pores in four parallel rows, as shown in the figure. The anterior row of oval areas stretch across the groove dividing the 41st and 42nd annuli; the posterior row, across the groove dividing the 42nd and 43rd annuli; so that two rows of pores are associated with the 42nd annulus, while the 41st and 43rd have each one row of pores. Six annuli intervene between the female orifice and the glandular area.

Leidy[2] has suggested that the glands opening through these pores are "provided for the adherence of individuals in sexual intercourse," and their position favours this view.

Ocelli.—Five pairs; arranged precisely as in Hirudo.

Œsophagus=about one sixth of the length of the Leech. The number and arrangement of the folds, so far as I could learn from the specimen examined, agreed very nearly with Leidy's description.

Maxillæ.—Three, large; armed with thirty-nine to forty-six acute and slightly curved denticles.

[1] V. K. Brooks, 'Handbook of Invertebrate Zoology,' Boston, 1882.

[2] Leidy, 'Proc. Phil. Acad. Nat. Sci.,' 1868, p. 230.

Nephridia.—Seventeen pairs,[1] located in the same rings as in Hirudo.

Segmental Papillæ.—Beginning with the 5th annulus, which bears the fourth pair of eyes, the papillate annuli are,—5th, 8th, 11th, 14th, 19th, 24th, 29th, 34th, 39th, 44th, 49th, 54th, 59th, 64th, 69th, 74th, 79th, 84th, 89th, 94th, 98th and 100th. Occasionally we find traces of pappillæ on the 102nd. Thus the number of papillate annuli is the same as in Hirudo; but the order is the same only as far as the 94th, which shows a difference in the annular composition of the posterior abbreviated somites.

Each of these annuli, except a few at either end of the body, bears fourteen minute segmental papillæ, eight on the dorsal and six on the ventral half. On the dorsal side (figs. 57, 59, Pl. XX) there are two median (*m.*), four lateral (*il. ol.*), and two marginal (*mg.*); on the ventral side (fig. 58) two median, two lateral, and two marginal.

The median dorsal papillæ, which are smaller and less distinct than the others, can be traced as far forward as the 2nd annulus, being replaced on the 1st by the first pair of eyes. The two inner lateral papillæ (*il.*) are the most strongly developed, and exhibit very plainly a serial relationship with the eyes. The outer lateral papillæ could not be traced farther forward than the 5th annulus. Both the inner and outer lateral papillæ are whitish, and easily seen with naked eye. The marginal papillæ lie in the very edge of the olive green of this side, and are quite conspicuous from having the bright colour of the ventral side. No distinct traces of these were found anterior to the 11th annulus.

The median papillæ of the ventral side are extremely minute, and much farther apart than those of the dorsal side (fig. 58). The lateral papillæ lie directly behind the nephridial pores, and are almost as conspicuous as the pores themselves. Their distance from the margin is about one fourth of the

[1] Brooks states that there are eighteen pairs in the Macrobdella he examined.

width of the body. The marginal papillæ are very close to the edge of the body, and nearly as large as the lateral papillæ.

Colour.—The ventral side is a bright reddish brown, with a few small scattered flecks of black. The ground-colour of the dorsal side is dark olive green. The most conspicuous markings of this side are the median row of orange-coloured spots. The first of these spots forms an elongated patch, beginning on the 1st annulus and stretching back to the 5th. The hind end of this patch is almost constricted off, so that it sometimes appears to represent an independent spot. The entire patch can be regarded as four coalesced spots. Behind this there are twenty of these spots, one on each papillate annulus as far as the 98th. The 100th annulus had no orange spot in any of the specimens examined, but it does have it in some other species. These spots have an elongated form, except when the Leech is much contracted, each one stretching across a papillate annulus and a half or more of the annulus following. The elongation in an antero-posterior direction suggests that they are remnants of a median stripe, which was once continuous over the non-papillate as well as the papillate annuli. The presence of small flecks of yellow scattered sparingly along the median line can be most naturally explained on this hypothesis. Small dark flecks are thickly strewn along the median dorsal area; and these are perceptibly darker on each side of the orange spots, which may be taken as an indication that the hypothetical median stripe had dark borders.

The next most prominent markings are two rows of quadrangular black spots (6), one on each side, considerably nearer the margin than the median line. These spots lie between the lateral papillæ of each side, and are limited, for the most part to the papillate annuli; occasionally, however, they show a posterior elongation, more rarely an anterior one.

Just inside the black spots there is, on each side, a row of faded black spots, irregular in shape, and plainly forming parts of an obsolescent dark stripe.

The very narrow margins of the dorsal surface have the colour of the ventral side.

Habitat.—Found in the neighbourhood of Cambridge; geographical limits unknown.

Abbreviated Somites.—There are twenty-six somites, of which the first six and the last four are abbreviated. The abbreviation at the anterior end agrees with what has been seen in Hirudo. The 24th, 25th, and 26th somites have each two annuli or remnants of annuli, and in so far agree with the Hirudo type. In the 23rd somite we find an important difference between the two genera; for in this somite there are at least four annuli in Macrobdella against three in Hirudo. The second annulus of this somite (95th in fig. 59) must be regarded as two annuli in process of consolidation; as its two halves show a well-marked separation at one (left in the fig.) and sometimes both margins. The two halves are, together, only a trifle thicker than the 94th annulus; but they are much thicker than the 96th. This peculiar double annulus is found in several (perhaps all) other species of Macrobdella. The process of abbreviation has only fairly begun in this somite, and has just reached a point that leaves it doubtful whether we have four or five annuli.

Differential Characters.—The genus Macrobdella is distinguished from Hirudo by the following characters:

1. Copulatory glands.
2. Four (or five) annuli in the 23rd somite.
3. Neither the buccal nor the post-buccal annuli are united on the ventral side.
4. Cephalic lobe smaller.

It remains to be seen whether this genus may be subdivided according to the number of rings separating the sexual orifices.

Leptostoma pigrum, g. et sp. nov. Pl. XVIII, figs. 21—27.

Diagnostic Characters.

Body large and fleshy, tapering towards the head more rapidly than in Hirudo (figs. 22 and 23).

Length of one of the larger specimens, swimming, 16·5 cm.; in extension, 21 cm.
Width „ „ 2 cm.; at rest, 2·5 cm.

Cephalic lobe, as in Hirudo, except much smaller proportionally.

Acetabulum 8 mm. in diameter, relatively smaller than in Hirudo.

Annuli 106.

Buccal annuli=5th and 6th. The coalescence is quite complete in the middle of the ventral side, but towards the margins they are distinct.

Post-buccal annuli=7th and 8th. Generally distinct on the ventral side, but not so deeply separated as the succeeding annuli.

Genital Apertures.—Male orifice between the 32nd and 33rd annuli, two annuli behind the fourth pair of nephridial pores. Female orifice between the 37th and 38th annuli.

Clitellum embraces the 9th (except 1st annulus), 10th, and 11th somites, and one annulus of the 12th somite, making fifteen annuli (twenty-seven to forty-one inclusive).

Anus in the last annulus (106th).

Ocelli, five pairs, arranged as in Hirudo. First pair the largest.

Œsophagus relatively long; with six folds, one dorsal, one ventral, two dorso-lateral, two ventro-lateral. The dorsal and ventro-lateral terminate in the maxillæ. The dorso-lateral folds small at the level of the maxillæ, but larger posteriorly.

Maxillæ three, small, on alternate folds, destitute of proper denticles, but provided with two series of irregular, thin, denticular plates, which are more or less united, especially at the outer and inner angle (*e* and *i*, fig. 62, Pl. XXI), where the two series bend into each other, thus completing the circuit of the outer edge of the jaw. These two series of brownish-yellow chitinous plates correspond to the double roots of the denticles in Hirudo; they rest on a thick muscular welt (*w.*, fig. 61), and are very feebly developed at the two angles of the jaw. In the elongated area, inclosed by the plates, numerous small fragmentary pieces of the same colour and texture are seen (fig. 62).

Stomach plainly divided metamerically, but the chambers much smaller than in Hirudo. The posterior chamber prolonged in two narrow lateral diverticula.

Intestine (stomach, Gratiolet) divided anteriorly into four chambers, the first of which is quite as wide as the chambers of the stomach.

Nephridia, seventeen pairs. First pair of pores in the hind edge of the 15th annulus; the seventeenth pair on the hind edge of the 95th annulus. Four pairs in front of the male orifice.

Segmental Papillæ.—Six dorsal and six ventral rows, as in H. nipponia. The papillate annuli, omitting the eye-bearing annuli, occur in the following order: 11th, 16th, 21st, 26th, 31st, 36th, 41st, 46th, 51st, 56th, 61st, 66th, 71st, 76th, 81st, 86th, 91st, 96th, 101st, 103rd, 105th (figs. 54, 55, Pl. XX).

The six dorsal rows are in pairs, one median pair and two lateral (fig. 27 *d*, Pl. XVIII); the ventral rows are arranged as shown in fig. 27 *v*.

Colour.—The dorsal side is brownish olive, with fine dark brown stripes, along each of which are placed, at regular intervals, oval or quadrangular yellow spots. The median stripe is usually darker than the lateral stripes, and in this the spots are sometimes wanting or much reduced in size (fig. 27 *d*). The inner lateral stripe runs midway between the median stripe and the margin, and the outer lateral stripe midway between the inner lateral stripe and the margin. In some cases (figs. 22, 24 *d*, 27 *d*) a shadowy stripe is seen on each side of the median stripe, equidistant from this and the inner lateral stripe; and this, in rare instances, may also be marked by yellow spots (fig. 24 *d*). The margins are usually bright orange yellow, bordered on the inner side with a narrow line of dull brown, or with mere flecks of this colour. The marginal yellow is continued round the acetabulum and the head.

The yellow spots of the median stripe become confluent anteriorly, forming thus an elongated patch which reaches to

the first pair of eyes. The spots of the lateral stripe sometimes blend in a similar manner.

The spots occur on the 2nd and 4th annuli of the somite, so that an interval of one annulus (3rd) alternates with one of two annuli (5th and 1st). Thus ten spots, two in each stripe, are found on each of the unabbreviated somites. This is the typical arrangement of the spots in specimens found about Tokio; but it is occasionally modified by the interpolation of small spots, as shown in figs. 22 and 27 *d*. In specimens obtained from a small lake (Junsainuma) near Hakodaté, in Yezo, the interval of one annulus is filled by a spot, so that three spots occur on successive annuli, followed by two annuli without spots (fig. 24 *d*). This arrangement may occasionally be modified by filling up some of the intervals of two annuli.

The entire absence of these spots in the median stripe of some individuals, the variations resulting from the filling up of the intervals, and their confluence at the anterior end of most specimens, all suggest that they may be regarded as remnants of yellow stripes, such as are seen in H. nipponia.

On the acetabulum one or more broad median patches of yellow are seen, which represent parts of the original stripe, or perhaps confluent spots. These patches are bordered laterally by a narrow wavy line of dark brown or black, precisely as are the spots on the body.

In many specimens dark flecks are scattered along each side of the median stripe (fig. 23).

The ventral surface is generally a dull orange yellow, marked with broad marginal stripes of dark brown with interspersed flecks of black, and with six or more narrow and much broken intermediate brown stripes. The ground colour of this side often varies towards the olive (fig. 27 *v*.) and brown shades (fig. 24 *v*.).

Genital Organs.—The penis lies on the left side of the nerve-chain, just behind the sixth pair of ganglia. The vagina, consisting of a saccular and a tubular portion (fig. 67 *v*.), lies on the right side of the nerve-chain, reaching from near the

7th to the 9th ganglia. The left oviduct passes under the nerve-chain, just the reverse of what happens in H. nipponia. The common oviduct (*od. c.*) and the gland (*gl. alb.*) adhere to the saccular portion of the vagina.

Habitat.—Ditches and ponds around Tokio and Yezo. None found at Aomori. Much less abundant than the common Medicinal Leech.

Habits.—Very sluggish; not easily induced to swim, though swimming well when forced. Food unknown; probably carnivorous.

LEPTOSTOMA EDENTULUM, g. et sp. nov. Pl. XIX, figs. 28—39.

Diagnostic Characters.

Body.—Small, tapering gradually to the very narrow head (figs. 28 and 29).

Length, swimming,	5·5 cm.;	in extension, 7·5 cm.;	abreast, 4·5 cm.
Width „	1 cm.;	„ 6 mm.;	„ 9·10mm.

The largest individual found measured, in extension, 12 cm.; swimming 8·5 cm.

Cephalic lobe and anterior portion of body extremely narrow.

Acetabulum 4 mm. in diameter.

Annuli 105, with sometimes a rudiment of a 106th behind the anus.

Buccal annuli = 5th and 6th, united on the ventral side.

Post-buccal annuli = 7th and 8th, united on the ventral side.

Genital Apertures.—Male orifice between 32nd and 33rd annuli—two annuli behind the fourth pair of nephridial pores. Female orifice between 37th and 38th annuli.

Clitellum 9th, 10th, and 11th somites.

Anus cuts the 105th annulus.

Ocelli five pairs, as in Hirudo.

Œsophagus has six folds, one dorsal, one ventral, two dorso-lateral, two ventro-lateral.

Maxillæ three, very small, only a little higher than the

folds to which they belong (fig. 63, Pl. XXI), showing absolutely no trace of denticles or rudimentary plates.

Nephridia seventeen pairs; first pair in the 15th, last pair in the 95th annulus. Four pairs in front of male orifice.

Segmental papillæ in six dorsal and six ventral rows. The papillate annuli have the same number as in the preceding species.

Colour.—The ground colour of the dorsal side is in the majority of cases a rich chrome green, sometimes exhibiting an exquisite shade of dark blue (fig. 28), more rarely inclining to the dull olive hue of fig. 39. There are five longitudinal stripes, one median and on each side two lateral. The median stripe is the broadest and most conspicuous, and is continuous from the first pair of eyes to the hind edge of the acetabulum. It is usually a brilliant chrome yellow, sometimes a gamboge yellow, or a bright golden yellow. It is bordered on each side by a narrow line of black, which is not sharply outlined.

The lateral stripes are always narrower, generally duller; and sometimes one or both of them (more frequently the inner one alone) are obsolete or obsolescent. As a rule, the lateral stripes, when present, are interrupted on every 5th annulus by the approximation of the black borders (figs. 33 and 37); and when absent are replaced by a black stripe, which represents the two borders united. These stripes are partially obliterated in fig. 28, wholly so in figs. 31 *d*, 32 *d*, and 34.

The narrow margins are yellow, generally paler than the median stripe.

The ventral side exhibits various shades of green and olive, more or less thickly sprinkled with black flecks. Alongside the yellow margins these flecks are so numerous that they might almost be said to form broad black borders.

A specimen from Aomori, the only one found in this locality, was very dark green, with a bright median yellow stripe and black lateral stripes (fig. 31).

Habitat.—Found in shallow pools in the rice fields around Tokio. Only one specimen found in Aomori, and none in Yezo. Comparatively rare.

Habits.—Active, easily provoked to swim. Food unknown.

Internal Organs.—The female organs (fig. 66, Pl. XXI) are in every respect similar to those of H. nipponia. The vagina is sometimes on the right, sometimes on the left of the nerve-chain.

The stomach is a narrow straight tube, with no distinct division into chambers, with two long slender diverticula at the posterior end. The intestine is divided into three regions of nearly equal length. The first is much wider than the stomach, and is divided into four chambers; the second is a narrow middle piece with a single coil; the third is a dilated fusiform end piece.

The dermal glands are larger and more numerous on the dorsal than on the ventral side.

The nephridial vesicles are large, and lined with a ciliated epithelium. The cilia appear to be absent in the region which leads into the efferent duct.

This beautiful little Leech agrees with a few forms found elsewhere in having no denticles; but this character appears to me to have comparatively little value as a generic distinction; certainly, much less than the number of its abbreviated somites, which links it with L. pigrum and L. acranulatum.

Toothless leeches have been found in various and widely-distant parts of the earth, which, so far as the descriptions go, differ from one another in important particulars. Philippi[1] describes a gigantic leech (?) from Valdivia, under the name of Macrobdella valdiviana, which has neither eyes nor jaws. Grube[2] has described a curious subterranean form (Cylicobdella lumbricoides), also without eyes or jaws. Cyclobdella glabra[3] is said to have ten eyes, but no jaws; and Hylobdella, Doringii, and H. flavolineata are reported

[1] Halle'sche, 'Zeitschr. f. d. gesammten Naturwissenschaften,' vi, pp. 435—442, 1872. Cf. 'Leuckart's Bericht,' 1872—1875.

[2] 'Arch. f. Naturgesch.,' pp. 87—121, 1871. 'Leuckart's Bericht,' 1870—1871.

[3] 'Boletin de la academianacional de ciencias de la repub. Argentina,' iii, pp. 231—244. According to 'Leuckart's Bericht.'

to have one pair of eyes and no denticles. Several species of Bdella have been said to be without denticles; but Peters,[1] according to Leuckart, affirms that denticles are present. In Leuckart's collection is a Leech labelled Bdella nilotica[2] from Port Natal. The œsophagus has six folds, and three small edentate jaws on alternate folds. Paired rudiments of denticular roots were found along each side of the median crest of the jaw. This Leech has the large oval segmental papillæ seen in H. saigonensis, H. maculosa (Singapore), H. javanica, H. multistriata (Ceylon), with the same inclination shown in fig. 56, Pl. XX.

Kinberg has described three species with "edentate maxillæ,"—D. decemstriatus from Montevideo, D. natalensis from Port Natal, and D. maculatus from Wisconsin.

Verrill[3] found "no distinct maxillæ" in Lemiscolex grandis, and mentions none in the case of Hexabdella depressa.

LEPTOSTOMA ACRANULATUM, g. et sp. nov. Pl. XIX, figs. 40—46.

Diagnostic Characters.

Body attains a greater length than in H. nipponia; general form and proportions are the same.

Length, swimming, 9-10 cm.;	in extension, 12-15 cm.;	at rest, 7-8 cm.
Width „ 10 mm.;	„ 7 mm;	„ 14 mm.

Cephalic lobe smaller than in Hirudo, but larger than in L. edentulum.

Acetabulum comparatively small, circular, 3 mm. in diameter.

Annuli 104, with sometimes a trace of a 105th behind the anus.

[1] 'Berl. Monatsber.,' 1854. 'Leuckart's Bericht.,' 1854-5, p. 359.

[2] Is not this Democedes natalensis, Kinberg?

[3] 'American Journal of Science,' iii, p. 136, 1872; 'Report of Com. of Fish and Fisheries,' for 1872-73, pp. 672, 673.

Buccal Annuli = 5th and 6th, united at the middle of ventral side, but distinct towards the margins.

Post-buccal Annuli = 7th and 8th, fully united below.

Genital Apertures.—Male orifice near the middle of the 34th annulus (4th of the 10th somite), three and a half annuli behind the fourth pair of nephridial pores. Female orifice in the 39th annulus (4th of the 11th somite).

Clitellum.—Limits not determined.

Anus behind the 104th annulus; sometimes cuts deeply into the hind edge of this annulus.

Ocelli, five pairs, as in Hirudo.

Œsophagus has six folds.

Maxillæ, three, on alternate folds, furnished with from ten to fifteen pairs of rudimentary denticular roots (fig. 45). In some cases the roots are united, the pair forming then a single transverse plate. In some individuals I found either no traces of rudiments or only a few scattered fragmentary remnants.

Nephridia, seventeen pairs, beginning in the 15th and ending in the 95th annulus; located nearer the middle than the hind edge of the annulus.

Segmental papillæ, in six dorsal and six ventral rows. The papillate annuli have the same number up to the 96th as in L. pigrum; the remaining three are the 100th, 102nd, and 104th, instead of 101st, 103rd, and 105th. This is accounted for by the coalescence of the 2nd and 3rd annuli of the 23rd somite (fig. 53). These two annuli are still distinct in L. edentulum, but they are not so strongly divided as the following annuli. The papillæ are extremely small (figs. 41 and 42) as in the two preceding species.

Colour.—The ground colour of the dorsal side is olive or olivaceous brown. There are five stripes, one median and four lateral. The broad median stripe is constant and often very conspicuous, owing to the metameric broadening of its black borders (fig. 41). This stripe is a pale olive or brownish olive, usually a lighter shade of the ground-colour itself. Its dark borders generally swell at regular intervals, as shown in Pl. XIX, fig. 41; but this peculiar pattern is often imperfectly

developed, as in fig. 44, and, in some cases, is scarcely more than indicated in shadowy and faded colours (fig. 46). The lateral stripes are narrower and duller, and often scarcely differ from the ground-colour, their position, then, being recognisable by their dark borders. These stripes are constricted at every annular groove, and sometimes quite interrupted at these points, the dark borders becoming confluent, and forming thus a chain of oval areas (fig. 41). The margins (embracing a narrow area on both the dorsal and ventral side) are orange yellow, olive, or brownish yellow bordered on each side by irregular dark brown flecks.

The ventral side is olivaceous, and sometimes marked by a few scattered flecks of dark brown.

Habitat.—Abundant in the rice fields and ditches about Tokio. Found also in Aomori, but not in Yezo.

Habits.—Active. Food not known.

Internal Organs.—The male organ opens between the 6th and 7th ganglia (beginning with the sub-œsophageal); the female between the 7th and 8th. The ovaries do not lie near the anterior end of the vagina, as they do in all the foregoing species, but have shifted their position to a point just before the 12th ganglia (Pl. XXI, fig. 64). Both the vagina and penial pouch are very long. The vagina is not plainly differentiated into a saccular and a tubular portion, but its posterior half is somewhat larger than the anterior. The oviducts are concealed by a large ovate glandular mass (*gl. alb.*), which lies diagonally across the nerve-chain, concealing the 10th ganglia. The common oviduct (*od. c.*) issues from the small end of the albuminiferous glands, makes a few bends and enters the hind end of the vagina (*v.*).

The vas deferens (*v. d.*) of either side passes into a coiled portion, the vesicula seminalis (epididymis), near the level of the ninth pair of ganglia, emerges in the form of a long trumpet-shaped portion (*d.*), the ductus ejaculatorius, which tapers gradually into the narrow terminal portion of the efferent duct. This terminal part of the seminal duct passes forward to near the sixth pair of ganglia; then, making a short

bend, runs back along the dorsal side of the penial pouch (*p.*), and enters the pouch near its hind end, passing first through the so-called glandulæ prostaticæ.

The stomach, or that portion of the alimentary canal corresponding to the "stomach" of Hirudo, is a straight tube, showing (in alcoholic specimens) no trace of metameric division, and terminating behind in two slender diverticula, the length of which was not ascertained. Just behind the junction of the diverticula with the main canal, the intestinal portion begins to enlarge; and a litle farther back it becomes smaller, tapering quite gradually to the very end. The intestine may be described as a fusiform canal, not differentiated, so far as I could see, by superficial examination into regions, and showing no evidence of metameric constrictions.

Segmental Papillæ.

Literature.—The segmental papillæ of the Leech have been noticed by a considerable number of naturalists; but no one, so far as I have been able to learn, has suspected that they were sense-organs. Ébrard,[1] who has described and figured them, gives us no information in regard to their structure, and entirely overlooked their serial relationship with the eyes.

Thomas[2] recognised two of these on the dorsal half of every 5th ring, and tried in vain to inject them.

Fermont[3] found six or eight of these on the dorsal half of every 5th ring, and pointed out the fact that the papillate rings follow immediately the rings in which the nephridial pores are located. "It is necessary," he says, "in order to see them well, to examine a large Leech which has been immersed in boiling water, after having been gorged with blood."[4]

[1] Ébrard, 'Nouvelle Monographie des Sangsues Médicinales,' Paris, 1857.

[2] Thomas, P., 'Mémoires pour servir à l'histoire naturelle des Sangsues,' Paris, 1806.

[3] Fermont, 'Monographie des Sangsues Médicinales,' Paris, 1854.

[4] The works of Thomas and Fermont are known to me only through Ébrard.

The dorsal papillæ were also noticed by Savigny.[1] In describing Hæmopis, he remarks: "On remarque sur le dos de cette espèce des points saillans et diaphanes, rangés transversalement, au nombre de six ou environ, sur certains anneaux; il y en a d'abord sur le neuvième et le douzième, puis sur le dix-septième, le vingt-deuxième, le vingt-septième, et ainsi de cinq en cinq jusqu'au quatre-vingt-douzième inclusivement, après lequel on en trouve encore sur le quatre-vingt quinzième et le quatre-vingt-dix-septième.

"Ces points brillans, qui correspondent précisément aux vingt paires de pores situées sous le ventre, ne sont point particuliers à cette Sangsue, ni même au genre Hæmopis; on les voit très-bien sur les Sangsues médicinales et officinales" (p. 116).

Ébrard (l. c. p. 95) has described the ventral as well as the dorsal papillæ, and has correctly stated their number:—"Ces points blanchâtres existent, tous les cinq plis transverses, au nombre de huit sur le dos, de six à huit sous le ventre. Les deux du milien du dos sont très-visibles à l'oeil nu chez plusieurs Sangsues de la Hongrie, du Levant, d'Espagne et de Géorgie (figs. 51, 33), dont la couleur du dos est noirâtre. Tous sont trés-apparents sur les Sangsues noires de la Bresse, de la Bretagne et sur celles de la Suède; ils constituent les taches blanches qui out été signalées chez ces dernières annélides par M. le professeur Wahlberg."

Gratiolet (l. c., pp. 12, 13) mentions only the median dorsal papillæ as whitish spots, which mark the 1st ring of each somite ("zoonite").

Serial Homology with the Eyes.—In the Hirudo, Aulostoma, and Hæmopis of Europe, as well as in Macrobdella of America, and the Hirudo of Japan, the segmental papillæ are quite small, especially towards the ends of the body, and hence a close examination is required to make out their true relation to the eyes. In the Land Leech of Japan they are more strictly papilliform, and proportionally larger

[1] Savigny, Jules-César, 'Système des Annelides, principalement de celles des côtes de l'Egypte et de la Syrie.'

than in any of the aquatic Leeches. In other Land Leeches they are also very strongly developed, though somewhat less prominent than in the Japanese species. In the Medicinal Leeches of Saigon, Singapore, Java, and Ceylon, they are much larger than in the European Hirudo, and their homology with the eyes is here clear and unmistakable.

The large Medicinal Leech of Saigon, which I shall call Hirudo saigonensis, is one of the most favorable objects for the study of the topographical relations of the segmental papillæ, and from it the accompanying diagram (fig. 1) has been constructed. The papillæ are indicated by black dots, and the eyes by larger dots. On the 11th, the 14th, and the 19th rings there are eight dorsal papillæ, and on the 8th only six; but here it is plain that the fifth pair of eyes (*oc* 5) occupy the places of two inner lateral papillæ (*il*).

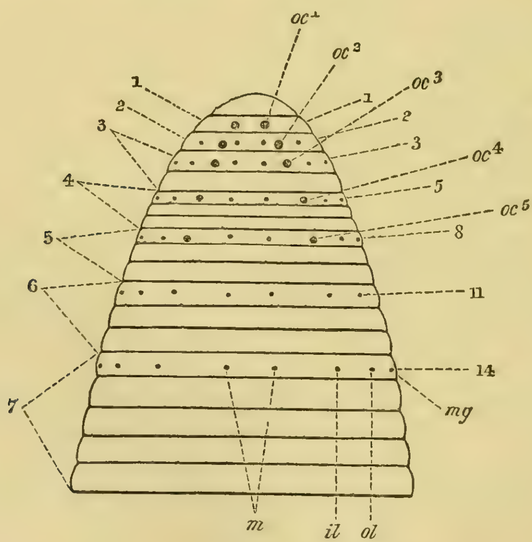


FIG. 1.—Diagram of first seven Somites of Hirudo saigonensis.—The figures at left of diagram indicate the somites; those at the right mark the first ring of each somite. *oc* 1—5. Five pairs of eyes. *m.* Median papillæ. *i. l.* Inner lateral papillæ. *o. l.* Outer lateral papillæ. *mg.* Marginal papillæ.

The same is true of the second, third, and fourth pairs of eyes. The first pair of eyes (*oc* 1) occupy the place of two median papillæ (*m.*), unless the appearances are deceptive. It is, of course, possible that the median papillæ of this ring have been lost, and that the eyes have developed from inner lateral papillæ. The appearances seem to me to favour the opinion that they have been derived from a pair of median papillæ. The papillæ are not round, but oval, and inclined as in Hirudinaria javanica (fig. 56, Pl. XX).

The median papillæ are arranged in metameric pairs, and the distance between the two rows is about the same as that between the first two eyes. Between the two rows of lateral papillæ on each side the distance is about half as great as between the inner lateral row and the median row. The marginal papillæ are placed at the extreme edge of the body. The outer lateral papillæ (*ol.*) are not recognisable on the first ring, and the marginal papillæ are absent on the 1st and 2nd rings; but their presence on the remaining rings (3rd, 5th, and 8th) makes it plain that all the eyes, except perhaps the first pair, occupy the place of the inner lateral papillæ.

Structure.—In comparing the Land Leech with the Medicinal Leech, I have already described the structure of the segmental papillæ. Sections of Macrobdella throw some light on the nature of what I have called the "white corpuscle" in the large clear cells which form the central portion of the eye, and which are associated with the segmental papillæ and with the "goblet-shaped" organs of the lip.

Fig. 71, Pl. XXI, shows two of these cells from the eye. In one of these the "white corpuscle" appears in the form of three bubble-like vesicles or vacuoles. In some cells I find as many as six of these spherical vacuoles, each bounded by a thin but distinct film. These spaces contain a watery fluid which does not stain in the least. The protoplasm of the cells is granular, and forms a peripheral layer, thickened on one side, as shown in Leydig's figures. In this thickened portion which projects into the vacuolar space, may be seen a small oval area, somewhat more darkly shaded. The outline

of this area is not very sharp. Possibly it represents the terminal portion of a nerve, but I have obtained no evidence in support of this view.

The small oval or elliptical nucleus (*n.*) is usually found at the base of the thickened portion of proptoplasm.

Function.—Ébrard ventures the following suggestion as to the function of the segmental papillæ. "Il se pourrait que ces parties fussent les analogues rudimentaires des houppes respiratoires ou autres que plusieurs des annélides dorsi-branches, je citerai les amphinomes, portent sur chacun des anneaux du corps."

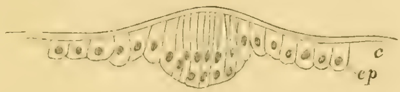
I have shown that they are sense-organs, and that from them the eyes have developed. I have not discovered any sense-hairs belonging to these organs, but I have found that a branch of the lateral nerves runs to each of them. For reasons before mentioned, I think it probable that they represent incipient eye-spots.

Postscript.

The unavoidable delays that have prevented the earlier publication of this paper have afforded time for a renewed study of the segmental sense-organs; and the results obtained enable me both to enlarge and to modify to some extent my general conclusions on their function. These conclusions, as presented in the foregoing pages, were based first of all on the serial homology of the segmental sense-organs with the eyes, and second on their structure as ascertained from sections of the Land Leech. A study of these organs in Clepsine has thrown new light on their structure in Hirudo and closely-allied genera. By the aid of a few diagrams I shall endeavour to make clear their more prominent features in both classes of Leeches, and shall then offer a few further considerations relative to their function. I find only six distinct rows of segmental sense-organs on the dorsal side of Clepsine, corresponding to the median, inner lateral, and outer lateral of Hirudo.

On the ventral side, where they are much smaller and more

simple in structure, I have not been able to distinguish with certainty more than four rows, but think it not improbable



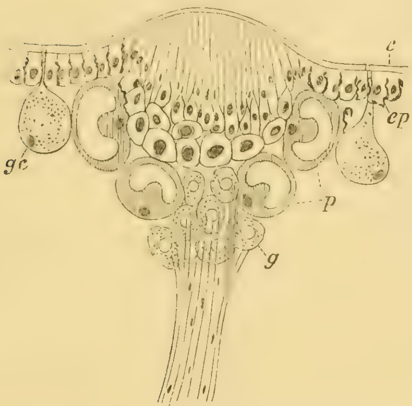


FIG. 2.—Section of one of the marginal sense-bulbs on the ventral side of Clepsine. The nerve and the large clear cells are not represented.

FIG. 3.—Diagrammatic section of one of the inner lateral sense-bulbs of the dorsal side of Clepsine.

c. Cuticle. *ep.* Epidermic cells. *gc.* Gland cells of the epidermic layer. *g.* Nerve-ganglion cells. *p.* Large clear cells, similar to those found in the eyes.

that more careful searching, assisted by sections, may bring to light two more. Fig. 2 gives a section of one of the organs placed very near the margin on the ventral side, and Fig. 3 represents a constructed section of one of the inner lateral sense-bulbs of the dorsal side. The organs of this row are not only larger, but more highly developed than those of the other rows.

The relative prominence of a single row of these organs on each side, so well marked in the Clepsine I have examined,

indicates a correspondingly higher functional importance. Carry this disparity in development and functional value to the extreme, and the result would be a single series of lateral-line organs on each side, as in the case of the Capitellidæ (Eisig). The presence of several rows equally developed on each side appears to me to represent an earlier condition than that of a single row, since it is more easy to account for the disappearance of one or more rows than to explain their independent origin in animals that have had a common derivation. Assuming that some ancestral form possessed several series of lateral-line organs, we should naturally enough expect to find variation in the number of series preserved in derived forms, some perhaps preserving all, while others preserved only a part or none at all. This view seems to me the most satisfactory way of accounting for the occurrence of more than one series of lateral-line organs in the Amphibia and some Fishes.

The structure of the segmental sense-organs of Clepsine is fairly shown in Figs. 2 and 3. The organ represents a bulb-like thickening of the epidermis, supplied with a branch of the lateral nerve of the corresponding body segment. The outer face of the bulb rises as a rounded prominence above the general surface; the inner, more strongly rounded face is cushioned in the connective tissue that intervenes between the epidermis and the ring muscles. Imbedded in this connective-tissue receptacle are a number (four to eight) of very large clear cells (*p.*), differing in no respect from the large cells found in the eyes. These cells are loosely placed around the bulb and nerve, and often one or more of them may be seen at a little distance from the bulb, either below it, alongside the nerve, or to one side. I have nothing to add to what is known about the structure of these peculiar cells, except that they are nucleated (a point disputed by Ranke). I regard them as the morphological equivalent of the epidermal gland-cells (*g. c.*), and therefore as belonging primarily to the epidermis. At the base of the bulb, often extending to a greater depth than shown in Fig. 2, are to be seen in most of my preparations some rather

large rounded cells (*g.*) which appear to be ganglionic in nature. The peripheral cells of the bulb are densely packed, thread-like cells, with pyriform inner (nucleated) ends. The terminal portions of these cells present a rod-like appearance in the apical region of the bulb, and are here more highly refractive than elsewhere. The cuticle extends over the whole external surface of the bulb, but becomes very thin over the circular apical area which is marked by the refractive rod-like ends of the sensory cells. So far as I have been able to learn, these elongated peripheral cells of the bulb are never prolonged beyond the cuticle. The same rod-shaped, refractive end portions are seen in the goblet-shaped sense-organs of the lip and in the eyes.

In Hirudo and Hæmadipsa these organs have the form seen in Fig. 4. The same elements enter into the composition of the organ. The sense-cells are more elongated, and often collected in small groups, to each of which runs a distinct branch of the nerve.

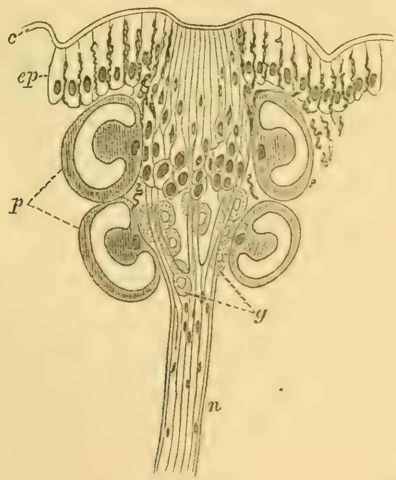


Fig. 4.—Section of one of the inner lateral sense-organs of Macrobdella in a state of retraction. *c.* Cuticle. *ep.* Epidermic cells. *g.* Ganglion cells. *p.* Large clear cells. *n.* Nerve-fibres.

The so-called goblet-shaped organs of the lips differ from that seen in Fig. 4 only in being more strongly developed and in having no large clear cells around the peripheral sensory

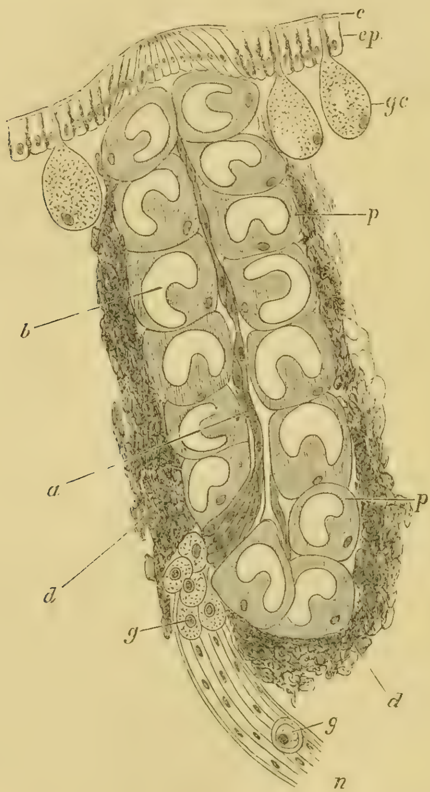


Fig. 5.—Diagrammatic section of the eye of the Land Leech. *c.* Cuticle. *ep.* Epidermic cells. *gc.* Gland-cells of the epidermic layer. *g.* Nerve-ganglion cells. *d.* Pigment. *n.* Nerve-fibres. *p.* Large clear cells. *a.* Nuclei of the same. *b.* Refringent substance of the same.

cells. I have found, however, some of these peculiar cells along the nerve-branches running to these organs.

With respect to the structure of the eye and the morphological significance of the elements composing it, my studies

lead to conclusions fundamentally different, in some important particulars, from those reached by Leydig and Ranke. It is not my intention to deal with details of history and criticism here, and I shall only call attention to points of special interest and importance in forming a correct notion of the eye. In passing, it may be worth while to call attention to some of the figures given by the above-named authors, in order to show wherein they are, in my opinion, misleading. In the first place, Leydig, whose figures are by far the most instructive of any that have yet been published on this subject, describes the sense-organs of the lip and head as goblet-shaped ("becherförmige Organe") organs with a shallow rounded cavity opening at the peripheral end. This cavity, which is only a depression resulting from retraction of the organ (see Fig. 4), is about the only justification for comparing these organs to a goblet. In a state of functional activity, all the sense-organs of the Leech are protruded, so that the peripheral end forms a convex surface (Figs. 2, 3 and 5) as was stated long ago by E. H. Weber. This cup-shaped depression of the retracted organ was supposed to be open at the bottom, the epidermic wall of the cup having a central circular perforation, in which the optic nerve terminated "unbedeckt." The optic nerve, penetrating the eye at the base, is represented as an "Achsenstrang" running the entire length of the eye. Placing the eye so that he could look directly into the cup-shaped depression, Leydig saw, through the supposed opening at the bottom of the depression, a peculiar spot somewhat broader in extent than the "axis-string" seen in transverse section. In preparations treated with reagents, this spot presented a granular aspect, while in a fresh condition it appeared to be composed of "glänzenden Kügelchen," which represented the terminations of nerve-fibres. Ranke gives a diagrammatic section of the eye, in which he leaves the epidermal cover entirely away, and says nothing about a central perforation. Now the peculiar spot seen by Leydig is probably the apical area seen in Fig. 5, in which the central cells of the epidermic cap present refractive rod-shaped ends. This interpretation is the only one

which appears to me to reconcile Leydig's fig. 2[1] (pl. iii) with an actual longitudinal section of the eye.

Ranke's fig. 8[2] (pl. x.) presented one feature that should be noticed here, namely, a ganglion opticum, placed not at the base of the eye, but near its external end.

Between the so-called ganglion and the epidermal cap of the eye only two layers of the large clear cells (P. in my fig.) intervene, while as many as eight lie between it and the base of the eye. The large clear cells in front of the ganglion are supposed to function as a cornea and lens, and to throw images of external objects on the retinal area ("ganglion opticum"). I find nothing in my sections at all comparable with Ranke's optic ganglion, unless it be the axial fibres seen in section. With the ganglion placed near the peripheral end of the eye, as in Ranke's figure, and on the supposition that the large clear cells which lie in front of it serve the purpose of a cornea and lens, the great mass of these clear cells lying behind the ganglion would appear to be useless. This fact alone invalidates Ranke's interpretation and lends some weight to the suggestion that his ganglion opticum was only a sectional view of the axial fibres.

The point on which I differ most widely from Leydig and Ranke lies in the interpretation of the axial fibres of the eye. I regard these fibres as very much elongated sense-cells, derived primarily from the epidermis, and in no sense of the word representing nerve-fibres. My reasons for this view are briefly the following: 1. The optic nerve is at least three times as thick as the widest place in the axial cord of fibres. 2. In a preparation treated with chromic acid twelve hours, washed in water twelve hours, gold chloride one hour, formic acid forty hours, I find that the optic nerve has a decided pinkish colour, while the axial fibres of the eye are stained blue, like the large clear cells and the epidermal cells. These two facts show quite conclusively that the axial fibres are not a direct continuation of the optic nerve.

[1] 'Tafeln zur Vergl. Anat.,' Tübingen, 1864.

[2] 'Zeitschr. f. wiss. Zool.,' xxv, 1875.

Comparing now the eye with one of its serial homologues, a segmental sense-organ, we find that the axial fibres occupy the same position with relation to the nerve and the large clear cells as the sensory cells of the segmental sense-organ. What is more natural than to regard the axial fibres as the sensory cells of the eye? I have sections in which the sensory cells of the segmental sense-organ could scarcely be distinguished if placed side by side with the axial fibres. Nuclei are seen along the axis of the eye, which appear to occupy the enlarged ends of the axial cells. This is best seen in deeper cells, which appear to be continuous with the fibres of the optic nerve. In none of my sections have I been able to trace the axial fibres (or cells) up to the epidermal cap, but I do not think it certain that they do not reach the shorter central cells of the cap. If they are completely separated from the epidermis, this would not of course be any obstacle in the way of accepting the view I have presented.

In the epidermal cap it is necessary to distinguish a central or *apical area* of relatively short and nearly perpendicular cells from a *border ring* of longer and strongly convergent cells. The cells of one area pass insensibly into those of the other, the length and degree of convergence increasing from the centre outward, so that they cannot be said to be sharply defined. The short, refractive, rod-like terminations of the central cells to which attention has already been called, enable one, however, to distinguish quite easily the two areas. In a retracted state the cells of the outer area, or border ring, are strongly inclined towards a horizontal position; and when seen from the surface they appear to radiate from the central area precisely as they are represented in Leydig's figures.

The central area then corresponds to what Leydig mistook for a perforation of the epidermal cap, in which the axis-fibres terminated.

From this point of view the eye appeared to be a sac-like invagination of the skin, in which the epidermis was represented by an inner wall of large clear cells and the corium by a thin limiting membrane ("sclerotica") and a thicker pig-

mented layer ("chorioidea"). This conception of the eye was rendered all the more plausible by the supposed central perforation of the epidermal cap, which remained permanently open, while the rest of the lumen of the sac was filled by the axial fibres. It was thus that Leydig maintained that the large clear cells were modified epidermic cells, an opinion in which Ranke fully concurs. According to the view I have presented, the eye is a solid ingrowth of dermal elements, the epidermis being represented by an axial cord of sensory cells continuous at the base of the eye with the optic nerve, the gland-cells of the skin by the large clear cells forming the bulk of the eye, and the sub-epidermal connective tissue by the pigment layer. I have not been able to satisfy myself from my sections that a distinct membrane-like layer (Leydig's sclerotica) separates the pigment investment from the large clear cells. It remains to be seen how far this view represents the actual developmental history of the eye.

Structurally considered, we are able to distinguish at least three different classes of sense-organs in the Leech. The first class embrace the segmental sense-organs of the body and head, and the non-segmental sense-bulbs scattered over the upper surface of the head; the second is represented by derivatives from segmental sense-organs, the eyes; and the third includes the goblet-shaped organs of the margin of the lip. In the first and third classes a bulb-like thickening of the epidermis forms the larger part of the organ, and the chief distinction between them lies in the presence or absence of large clear cells around the bulb. The distinguishing feature of the second class is the massive development of the large clear cells.

With respect to arrangement all these organs may be grouped in two divisions, one of which is strictly segmental, the other non-segmental or accessory. All agree in representing primarily more or less specialised portions of a common morphological basis, but the bilateral and metameric symmetry of one set of organs must be regarded as a distinctive feature of considerable significance. For while the source of origin is

certainly the same, the time and conditions which brought the two sets of organs into existence cannot be identical in all respects.

In respect to time of origin, the segmental sense-organs must be placed first, for the non-segmental organs are limited to a specialised part of the animal, and have undoubtedly arisen in response to the increased needs of this part. There is not the slightest reason to suppose that they owe their origin to a multiplication of the segmental sense-organs by division. On the contrary, it seems quite certain that they must have arisen quite independently.

Before considering the question of function, there are a few points of comparison to be noted between these organs and the lateral-line organ of the Fish.

In the Teleostean Fish these organs pass through, in their early development, a stage which is identical with the simple epidermal thickening that remains permanent in Clepsine (see Fig. 2). In a somewhat later stage (Fig. 6) the peripheral cells develop hair-like extensions, which coalesce to form a

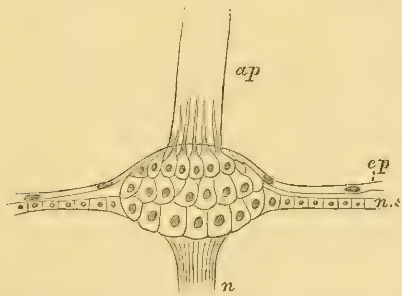


FIG. 6.—Lateral-line organ of a Teleostean Fish at the time of hatching. *ap.* Strap-shaped appendage. *ep.* Epidermis. *n.* Nerve. *n. s.* Nervous stratum.

delicate strap-shaped appendage. The addition of such a mechanism for raising the sensory power of the cells in a special direction does not of course, in view of the facts now well known in regard to the morphology of sense-organs,

make it any the less probable that the lateral-line organs are homologous with the segmental sense-organs of the Leech.

In respect to the nerve supply of these organs, a modification of its segmental character has been brought about in the head of the Leech analogous to what is seen in the body of the Fish. As is now well known, the lateral-line organ of the head of the Fish are each supplied with a segmental nerve-branch, while those of the body are supplied with branches from a single lateral nerve. In the Leech the segmental sense-organs of the body are each supplied with a segmental nerve-branch, while in the head (cf. Leydig's fig. 5, pl. ii) we find a single nerve sending branches to two or more segmental sense-organs, and the same nerve supplying one or more pairs of eyes and numerous goblet-shaped organs of the lip. In this latter particular we have a good illustration of the fact that nerves are not functionally differentiated according to the different sense-organs they supply.

The facts here presented appear to warrant the opinion that at least three different functions are represented in the sense-organs of the Leech. The evident serial homology of the eyes with the segmental sense-organs, and the presence of large clear cells in both classes of organs, suggest that the different sense-organs may not be limited to the exercise of a single function. This view has been put forward by Ranke on the ground that the different sense-organs have been derived from a common morphological basis. It must be admitted that they originally exercised one or more functions in common; and their structural differences, while indicating plainly that they have made some progress in the direction of specialisation, are not so great as to exclude the possibility, or even the probability, that they are still able to do several kinds of work in common. With Claus I regard the goblet-shaped organs of the lip as organs of taste; but it seems almost certain that they function also as tactile organs, as was maintained by Leydig. When blood is placed in contact with the lip of the Leech its behaviour plainly indicates that it has the power of taste. In creeping about the lip is protruded, and the margin,

in which the goblet-shaped organs are located, is plainly used as an organ of touch.

I have never seen any evidence that the eyes are employed either for taste or touch, and the observations of Ranke on this point appear to me to have little value. If the structure of the eye is what I have represented it to be, it is plain that it is not adapted for receiving images of external objects. At most there can only be the power of distinguishing light from darkness, and this power the Leech certainly possesses.

There is still much uncertainty respecting the functional nature of the segmental sense-organs. It is quite certain, however, that they do not serve the same purpose as the lateral-line organs of the Fish or the segmental sense-organs of the Capitellidæ. This point is made certain in two ways; first, by the absence of sense-hairs, and second, by the fact that Land Leeches are provided with these organs. It seems also quite clear that their chief function is not that of tactile organs, for they are not more sensitive to touch than other parts of the epidermis. Their structure is in some respects much like that of organs of taste, but they certainly could not serve the Land Leech in this capacity. Excluding then the three senses of touch, taste, and hearing, there remain those of sight and smell, both of which would be very useful to both land and aquatic Leeches. The eyes are undoubtedly visual organs, but they alone are not sufficient for the obvious needs of the Leech. One of the most characteristic habits of Leeches in general is that of keeping themselves in dark or shaded places. It is not enough to screen the head from the light; the whole body, including the posterior sucker, require to be so protected in order to satisfy fully the usual conditions of rest. This is particularly true of Clepsine and Hirudo, and only a little less so of Nephelis.

But how is it possible for Leeches to know when these conditions are fulfilled for all parts of the body? This question is answered, if the segmental sense-organs are capable of distinguishing light and darkness. The massive development of the large clear cells in the eyes is very conclusive evidence that

their special function is more or less intimately associated with the work performed by the eyes. Although I do not feel prepared to adopt without reserve the opinion that they represent simply a dioptric mechanism, I think their presence in the eyes and the segmental sense-organs furnishes good ground for thinking that both classes of organs have a common function. Add to this their serial homology and the evidence becomes stronger in favour of the view maintained in this memoir respecting the function of the segmental sense-organs.

But how are we to explain the presence of the same large clear cells along the nerve-branches running to the goblet-shaped organs of the lip? They are not here associated with the peripheral sensory cells, as they are in the eyes and the segmental sense-organs, and I am not certain that they are constant. All that I can say is that they are to be seen in some of my sections of the Land Leech, and I confess to being quite unable to offer any explanation of them in this position.

While still maintaining that the segmental sense-organs, as well as the non-segmental sense-bulbs scattered over the upper surface of the head, share in the work of the eyes, I am strongly inclined to think that this is not their only work. In the case of the Land Leech I have obtained some evidence of a sense of smell. A breath thrown into the bottle containing these Leeches instantly puts them into a state of very great excitement. They move about in great haste, as if aware of the presence of some object tempting to their appetite. Any jar of the bottle is sufficient to excite them, but disturbance of this kind, however violent, falls far short of giving the stimulus imparted by a gentle breath. In removing specimens from one bottle to another I have often found a few of the less hungry ones disinclined to accept any opportunity to leave the bottom of the bottle. In such cases, when all other expedients failed to bring them out, I have found that breathing upon them soon induced them to come to the mouth of the bottle.

My observations on the habits of Clepsine marginata were made before my attention was directed to the question here considered; but, so far as my recollection serves me, I

should say that these Leeches are able to distinguish between a frog and a fish without being brought into contact with them.

This Clepsine is a fish parasite, and would be a favorable object with which to test this question.

I have made some experiments with one of our large pond Leeches (Macrobdella) for the purpose of ascertaining, if possible, the function, or functions, subserved by the different sense-organs. I have not been able to settle the main question, but the results are perhaps worthy of brief notice. The experiments were as follows:

1. The muddy bottom of a pool inhabited by these Leeches was shaken and stirred up by walking through it. This disturbance aroused the Leeches, and set them to swimming about in search of the intruder. I watched for any signs of method in their attempts to find me; and in various ways tried to find out if they were able to guide their course by a sense of sight, of smell, or of touch. The experiment was made in rubber-boots, on a bright sunny day; and, after starting the Leeches in the manner described, I remained quiet and observed the result. More than fifty Leeches made their appearance in the course of an hour. They swam about in all directions, the number coming towards me not exceeding those taking any other course. They sometimes halted, coming to rest on some plant, and then started up the moment the water was again disturbed. While on the move they generally kept at the surface, often throwing the head slightly above the surface; and when coming to rest they assumed an attitude, not of repose but of watchfulness, as if waiting for fresh evidence of my presence. I am not fully satisfied that their course was directed wholly at random, but I was unable to get any satisfactory evidence that they were able to orient themselves with reference to the place from which disturbing waves proceeded. Waves were made, by the hand or foot, to strike them from the side and from the rear; but they called forth no intelligent response, and only in comparatively few instances induced a change of course. The change of course in answer

to such stimuli, whenever it occurred, appeared to be made at random rather than with a definite aim. Several times I held the finger just in front and a little to one side of the head; but the Leech swam on without turning to grasp it, even when held so near that the margin of the body grazed it in passing.

2. Thirty to forty of these Leeches were captured and placed in a glass basin, and left until they had become quiet. Then the end of the finger was quietly rubbed over a small area on the bottom of the basin, care being taken not to arouse the Leeches by any sudden movement of the water. After withdrawing the finger, the basin was moved just enough to set the Leeches in motion. They began at once to search about, some swimming, others creeping, or stretching at full length and swinging from point to point. If the expanded lip chanced to rest for a moment on the spot which had been rubbed with the finger, the Leech instantly showed unmistakable evidence that it tasted or smelled something agreeable, and began to examine the place with that quick and excited movement of the head which it shows when brought into direct contact with the finger. This behaviour must, I believe, be attributed to a sense of taste rather than smell, since it is not called forth except by actual contact with the lip. In the course of a few minutes several Leeches found the spot, and felt it over with as much delight as if it had been the finger itself.

3. A drop of fresh blood was allowed to flow from a pipette over the dorsal surface of a Leech while in a state of repose. The Leech kept up a gentle undulating movement of the body, and gave no evidence of recognition. As soon, however, as the blood flowed over the margin of the lip the Leech became aware of its presence.

This experiment, repeated many times, appears to me to show that the eyes and segmental sense-organs of Macrobdella do not function as organs of taste or smell.

EXPLANATION OF PLATES XVII—XXI,

Illustrating Mr. Whitman's Paper on "The Leeches of Japan."

PLATE XVII.

FIGS. 1—9.—Hæmadipsa japonica and H. ceylanica.

FIGS. 1 and 2.—Dorsal and ventral view of H. japonica at rest. Natural size.

FIGS. 3 and 5.—Similar views of another individual in extension, showing a different colour. Natural size.

FIG. 4.—Dorsal view of another individual, partially filled with blood (in extension). Natural size.

FIG. 6.—The anterior end of Fig. 1, magnified 10 diameters, showing the position of the eyes and their serial homology with the median and lateral segmental papillæ.

FIG. 7.—The posterior end of the same individual, magnified 5 diameters.

FIGS. 8 and 9.—Dorsal and ventral view of H. ceylanica. Natural size.

PLATE XVIII.

FIGS. 10—20.—Hirudo nipponia.

FIG. 10.—An outline figure of the Leech represented in Fig. 18, showing the whole number of annuli, the arrangement of the eyes and segmental papillæ, the distribution of the dark pigment, the position of the first (13th annulus) and last pair (93rd annulus) of nephridial pores, and the position of the genital openings (between the 30th and 31st and the 35th and 36th annuli). × 2.

FIGS. 11—13.—Fig. 11 is a dorsal, and Fig. 13 a ventral view of a middle portion of the Leech represented entire in Fig. 12. Figs. 11 and 13 are magnified 4 diameters.

FIG. 14.—A much faded individual, in which the five stripes are only faintly indicated. Natural size.

FIG. 15.—A dorsal (*d.*) and a ventral (*v.*) view of two middle somites of a Leech from Aomori.

Fig. 16.—Similar views of another individual from the same locality, in which the lateral stripes are nearly obsolete.

Fig. 17.—A middle-sized individual from Tokio, in which the inner lateral yellow stripes have been replaced by black stripes, each of which represents two dark borders united.

Fig. 18.—An individual in which the yellow stripes have all been replaced by black stripes.

Fig. 19.—A typically coloured specimen.

Fig. 20.—An unusually dark variety, in which the yellow stripes are continuous, or nearly so.

PLATE XVIII.

Figs. 21—27.—Leptostoma pigrum.

Fig. 21.—An outline figure showing the whole number of annuli, the precise arrangement of the yellow pigment spots, the position of the genital pores, rings embraced in the clitellum, and the number of abbreviated somites—five at the anterior end, and three at the posterior end of the body.

Fig. 22.—The same coloured. Natural size.

Fig. 23.—Another individual, which shows the typical arrangement of the yellow spots in specimens found in the neighbourhood of Tokio.

Fig. 24.—Dorsal (*d.*) and ventral (*v.*) view of the Yezo type. Specimen obtained from a small lake (Junsainuma) near Hakodaté.

Fig. 25.—A Tokio pattern, in which the median spots are absent.

Fig. 26.—Another, in which the spots are all very small, the median ones being almost obliterated.

Fig. 27.—A dorsal and a ventral view, showing the position of the segmental papillæ and the nephridial pores. × 3.

PLATE XIX.

Figs. 28—39.—Leptostoma edentulum.

Fig. 28.—Dorsal view of a large individual, slightly extended.

Fig. 29.—Ventral side of the same.

Fig. 30.—Dorsal and ventral side of ten middle annuli. Five stripes present; the lateral stripes narrow, and brownish yellow.

Fig. 31.—Similar views of a specimen from Aomori.

Figs. 32, 33, 35, and 36.—Similar views of Tokio specimens, showing different shades and patterns.

Fig. 34.—A small individual found near Nikko. The median stripe is reddish brown, the lateral stripes are replaced with black. Transverse dark lines mark the limits of the somites.

Figs. 37 and 39.—Two individuals from Tokio.

Fig. 38.—An enlarged view (× 2) showing the segmental papillæ of both sides, and the position of the nephridial pores.

PLATE XIX.

Figs. 40—46.—Leptostoma acranulatum.

Figs. 40, 43, 44, and 46.—Different patterns, taken from individuals found in Tokio. Natural size.

Fig. 41.—Dorsal side of ten annuli, enlarged (× 4) to show the segmental papillæ and the exact pattern of the colour-markings.

Fig. 42.—Ventral side of the same (× 4). The nephridial pores are about midway between the hind edge and the middle of the annulus.

Fig. 45.—Rudimentary denticles. × 165.

PLATE XX.

Figs. 47—59.—Diagrams illustrating the abbreviated somites in several genera of Leeches. As it is one of the designs of these diagrams to show the topographical relations of parts and organs to the papillate annuli, it seems advantageous to regard the objects as transparent bodies, in which the genital pores, nephridial pores, &c., of the ventral side may be seen in relation with the papillæ of the dorsal side. Remembering that the figures are constructed on this plan, no confusion between dorsal and ventral organs need arise. The annuli are numbered on the right of the figures, the somites on the left. The nephridial pores are indicated by the ordinals, the first pair being denoted by *1st p.*; the second, by *2nd p.*; &c. *m.* Two median papillæ. *i. l.* Inner lateral papilla. *o. l.* Outer lateral papilla. *mg.* Marginal papilla. *p.* Nephridial pores (1st—17th). *g. c.* Glandulæ copulativæ. *m. o.* Male orifice. *f. o.* Female orifice. *b.* Black spots. *y.* Yellow spots. *l.* Lateral papilla of ventral side. × Magnified.

Fig. 47.—Anterior end of Hirudo medicinalis, from Sweden. × 2.

Fig. 48.—Two rings seen from ventral side, showing position of papillæ, pores, and distribution of the black pigment. × 2.

Fig. 49.—Posterior end of the same individual, showing a fragment only of the 102nd annulus. × 2.

Fig. 50.—Posterior end of H. medicinalis, obtained from Sebeto River.

Naples. The anus is in the 102nd annulus, nearly dividing this into two parts. × 2.

Fig. 51.—Aulostoma gulo, auct., from Sebeto River, Naples. In this specimen (alcoholic) the papillæ were very distinct, the full number appearing even on the 2nd annulus. × 4.

Fig. 52.—Aulostoma gulo obtained in Leipsic. The anus is large, completely cutting the two small rings of the 26th somite. × 4.

Fig. 53.—Leptostoma acranulatum, from Tokio. The 2nd annulus of the 23rd somite is thicker than the preceding annulus or the following, thus appearing to represent the two annuli fused together; in this particular it agrees with Macrobdella of America. The 105th annulus is a mere rudiment, and is somewhat doubtfully regarded as belonging to the body. × 4.

Fig. 54.—Leptostoma pigrum, from Japan, showing only five abbreviated somites. The last ring (10th) of the 5th somite represents two rings consolidated. The first pair of nephridial pores is in the hind edge of the 15th ring, instead of the 13th as in the European Leeches. The arrangement of the stripes and spots, and the relation of the segmental papillæ to the stripes, are also shown. × 4.

Fig. 55.—The hind end of the same Leech, showing one full somite and three abridged somites behind the 17th pair of nephridial pores. The 106th annulus is completely cut by the anus. No marginal papillæ were visible. × 4.

Fig. 56.—Hirudinaria javanica.—(Hirudo javanica, Wahlberg), showing very large segmental papillæ with a definite inclination, which is repeated on every papillate ring. Their arrangement on the acetabulum shows that the eight or more somites, of which it is composed, are each represented by the papillate ring alone, all the non-papillate rings having been suppressed. The figure shows one of the ventral papillæ (*mg'*). The 102nd annulus forms a sort of neutral ground between the body and the acetabulum, and consists of two halves separated by the anus. The acetabulum is immense, reaching forward to the level of the last pair of nephridial pores (17th p.). × 4.

Fig. 57.—Macrobdella sestertia, obtained from Charles River, Watertown, Mass., shows the dorsal surface of the first twelve somites, and the position occupied by the organs of the ventral surface (nephridial pores, genital pores, and copulatory glands) (*gc.*). × 4.

Fig. 58.—Eleven rings seen from the ventral side, to show the position of the papillæ and the pores. The lateral papillæ (*l.*) are in line with the nephridial pores (*p.*). × 4.

Fig. 59.—The posterior end of M. sestertia, showing an abbreviation similar to that seen in Fig. 53 (Leptostoma acranulatum). × 4.

PLATE XXI.

Figs. 60—71.—Reproductive Organs and Maxillæ.

Fig. 60.—Median dorsal maxilla of Hirudinaria javanica, seen in profile. There are from 115 to 120 denticles decreasing gradually in size from the inner (inferior in the natural position) angle (*i.*) towards the external (superior) angle (*e.*). The maxilla is thicker than the fold (*f.*), to which it belongs; and its inner angle rises abruptly above the level of the fold. Rather large wart-like protuberances are seen on the lateral faces of the maxilla. × 50.

Fig. 61.—One of the maxillæ of Leptostoma pigrum, showing no denticles, but only two series of irregular flattened plates, which are more or less united, especially at the two angles (*i.* and *e.*) where they bend into each other, thus completing the circuit of the outer edge of the jaw. These chitinous plates rest on a thick muscular welt (*w.*). × 130.

Fig. 62.—An outline of the face of the jaw, showing that the plates are continuous, but weakly developed at the two angles (*i.* and *e.*). Numerous small irregular chitinous pieces are scattered throughout the area inclosed by the larger plates. × 130.

Fig. 63.—A profile view of the maxilla of Leptostoma edentulum. The jaw here is so small that it scarcely deserves the name; it is the anterior end of the fold (*f.*) slightly enlarged, and has no denticles nor any traces of even rudimentary plates. × 50.

Fig. 64.—Reproductive organs of Leptostoma acranulatum. The male organ opens between the 6th and 7th ganglia (counting the sub-œsophageal as one); the female organ between the 7th and 8th. The ovaries have shifted their position from between the 7th and 8th ganglia to a point just in front of the 12th ganglion. The vagina and penial pouch are extremely long. × 4. *t.* Testis. *vd.* Vas deferens commune. *vs.* Vesicula seminalis (epididymis). *d.* ductus ejaculatorius. *gp.* Glandulæ prostaticæ *p.* Penial pouch (sacculus penis). *o.* Ovaries. *gl. alb.* Glandulæ albuminiferæ. *od.* Oviduct. *od. c.* Oviductus communis. *v.* Vagina.

Fig. 65.—Female organs of Hirudo nipponia. Letters as before. × 4.

Fig. 66.—Female organs of Leptostoma edentulum. × 4.

Fig. 67.—Female organs of Leptostoma pigrum. The vagina differentiated into a tubular and a saccular portion. The common oviduct. (*od. c.*) adheres closely with the saccular portion of the vagina. × 4.

Fig. 68.—Female organs of Hæmadipsa japonica. Here the com-

mon oviduct has united with the saccular portion of the vagina, and thus appears to enter its anterior instead of its posterior end. × 4.

FIG. 69.—A maxilla armed with curved denticles, from Hæmadipsa japonica. × 50.

FIG. 70.—A maxilla with straight conical denticles, from Hirudo nipponia.

FIG. 71.—Two of the large central cells of the eye (Macrobdella). *n*. Nucleus. 1, 2, 3. Vacuolar spaces surrounded by the peripheral granular protoplasm. At one point the protoplasm thickens into a protuberance, which juts into the vacuolar space. × 465.

Contributions to the Embryology of the Nemertea.

By

Prof. A. A. W. Hubrecht,
Of Utrecht.

With Plate XXII.

I. The Development of Lineus Obscurus, Barr.

It is a well-known fact that the development of Lineus obscurus, first inquired into by Desor in 1848, is characterised by the appearance of a larval form, to which the name of Desor's larva has since been given. MacIntosh (17), Max Schultze (6), and Barrois (21), the latter more exhaustively, have since reinvestigated the development of this species.

Their researches were carried on by means of larvæ that had first been rendered transparent, i. e. by so-called optic sections. Having succeeded in making actual sections through all the different phases of development, and having ascertained that by the aid of this method we may obtain far more reliable data, I venture to return once more to the same subject, since I have convinced myself that the results made known by former observers are to a large extent incomplete, and must on various points be abandoned and replaced by such as are furnished by the more perfect modern method.

The outcome of my own investigations has already been fully published in the Dutch language,[1] in a treatise in quarto, printed

[1] 'Proeve eener ontwikkelungs geschiedenis van Lineus obscurus, Barrois, door Dr. A. A. W. Hubrecht, Prys verhandeling met goud bekroond en uitgegenen door het provinciaal Utrechtsch gerootschap van Kunsten en Wetenschappen,' Utrecht, 1885, 4to.

by the Utrecht Society for Arts and Sciences (30), and accompanied by six plates, in which the most important of the numerous sections through different stages have been figured. At the request of the Editor of this Journal I now give a full account of the contents of this more extensive paper, and a reproduction of the last of the six plates, in which the principal results are combined into fifteen diagrammatic tracings.

a. The earliest Developmental Stages and the Derivates of the Primary Epiblast.

Up to the stage when the invaginate gastrula has appeared (Pl. XXII, fig. 1) no difference obtains between Barrois's description (21) and my own. The hypoblast cells are larger sized than the epiblast cells, and at an early period, when the blastopore is still wide and spacious, the first traces of differentiation in the epiblast appear, which have escaped Barrois's notice, although the subsequent stages were again very correctly interpreted by him. I here refer to the formation of the discs of secondary epiblast. The first indication of the formation of these discs can be clearly traced in sections.[1]

At four different spots, of which two can only be seen at a time in transverse or longitudinal sections, we notice that the cubic epiblast cells divide lengthways, thus becoming palisade cells (Pl. XXII, fig. 2). No transverse division (delamination) is here noticed, such as we will have to describe by-and-by in other parts of the epiblast. When these four areas have definitely obtained this changed aspect the surrounding epiblast cells commence to overcap them (Pl. XXII, fig. 3), and thus they are very soon completely enclosed inside the primary epiblast. We have then the four discs so well known in the case of the Desor larva, and often compared to the four invaginate portions of the primary epiblast of Pilidium. I must, however, emphatically remark that the ultimate body wall of the young

[1] For the methods which I have followed in order to obtain these sections, and direct them according to a given plane, I must refer the reader to the Dutch treatise cited above (30).

Lineus does not arise out of four but out of five discs. The formation of the fifth disc commences but very little later than that of the four just mentioned; it, however, arises in a different manner. In the aboral region of the epiblast a very distinct process of delamination of the epiblast cells sets in (fig. 3), and the inner layer of cells remaining in connection with the outer (figs. 4, 5), a double layer thus originates, which finally separates, not, however, simultaneously along its whole surface, but first only in the middle (fig. 6), the dorsal layer of secondary epiblast being thus connected with the primary in the same manner as obtained in the paired lateral discs of secondary epiblast. All the five discs are one cell-layer thick; they increase in size by continued division of the constituent cells, and perhaps, also, by further participation of the primary epiblast along the line which marks the circumference of the discs; they finally meet along their edges; they then unite (fig. 9), and form the continuous coat of secondary integument, outside of which the primary epiblast is now only temporarily retained, and is very soon cast off.

Whereas we have seen that the fifth plate was decidedly dorsal in position, we might term the two lateral pairs the cephalic plates and the ventral plates. The former are situated before, the latter behind the blastopore (Pl. XXII, fig. 10), and a section through the blastopore (as in fig. 4) was often obtained, in which neither the cephalic nor the ventral pair of discs had been cut, and in which only the dorsal disc was visible. Horizontal (fig. 10) and longitudinal (fig. 7) sections reveal the presence of yet another centre of delamination in the primary epiblast, which is situated at the anterior pole. This delamination, which has been traced by me in all its successive phases (and more elaborately figured (30), pl. iv, figs. 55—59), is the first origin of the proboscis. It soon separates from the primary epiblast in its middle portion, which elongates and grows rapidly backwards (fig. 9), whereas the outer circumference fuses with the cephalic discs when these reach forward and meet along the median line (30) (pl. iii, figs. 43 and 44). From this moment it is no longer possible to perceive that the

proboscis arose, not as an inward growth of the secondary epiblast, but actually as an independent delamination in the primary epiblast.

We have now to note that the delamination process by which we have just seen that both the dorsal plate of secondary epiblast and the proboscis come into existence, is of a still greater extension, and that active delamination with a different purpose takes place not only in the epiblast but also in the hypoblast, even at still earlier stages of development. The delamination to which I now allude might more adequately be termed a budding of cells, the latter not forming a continuous "lamina." This budding process, by which independent cells are set free into the segmentation cavity—and which is no other than the formation of the mesoblast—commences in the early gastrula stage, and probably continues up to the time that the discs of secondary epiblast have coalesced to form the definite integument of the young worm. It was repeatedly observed in all its different phases, and fully figured (30) (pl. i, figs. 12—20; pl. ii, figs. 23—26, 33; pl. iii). There is no doubt that it affects both the epiblast and the hypoblast, and, secondly, that it is not definitely localised This process of proliferation, at the time it occurs, temporarily doubles those cells of the primary epiblast which undergo this transverse division; the epiblast, at a later period (fig. 2), is again only one layer thick (figs. 6—11). The hypoblast is only in its very earliest phases a distinct unicellular layer. Later on the cell walls become less distinct. Nevertheless, the budding of the hypoblastic mesoblast cells into the blastocœl was here traced quite as positively.[1]

[1] In an exceedingly able and suggestive paper on the "Development of Aulostoma gulo" ('Arb. aus d. Zool. Zoot. Institut zu Würzburg,' Bd. vii, p. 231), R. S. Bergh has, in addition to most valuable contributions to our knowledge of the ontogeny of the Discophora, instituted certain comparisons between these and the Nemertea. These comparisons are chiefly based on embryological data, and would indeed be more plausible if Barrois's account of the ontogenetic processes in Lineus could be relied upon. Now that closer investigations lead to the conclusion that the mesoblast in Lineus

These formative mesoblast cells, undoubtedly performing amœboid movements in the blastocœl, into which they have been set free, must now be studied somewhat more in detail. When once they are met with inside the segmentation cavity it is impossible to decide with certainty from which of the two primary cell layers each of them has taken its origin. Nevertheless, we can hardly doubt, on theoretical considerations, that certain differences of an essential hereditary nature must obtain between them. In tracing the origin of the nervous system we will further consider this problem. It may now suffice to call in mind the fact that of late years most important cytological researches have forced us to the con clusion that the nucleoplasma is of the highest importance in cell division, and that the life-history of certain cell groups, which, combined into distinct tissues, may be said to be determined by the nature of the nucleoplasm of the embryonic mother-cell from which they have all originated (cf. Weismann, 'Ueber die Continuität des Keimplasmas,' 1885). If this be true other hereditary determinants must be inherent in the epiblastic mesoblast cells than in the hypoblastic. A fact which must be considered in connection with this, and which probably has an important cytological significance (it was more fully described by me, l. c., p. 10), is that the chromatic nuclear substance of the primary epiblast diminishes, even when its surface increases, towards the time when it is going to be cast off. There is thus a decrease in the significance of the primary epiblast as a formative element for the moulding of the young larva inside it, which becomes more and more marked as the latter increases in size. Instead of holding that the nuclear substance which has disappeared from this

arises independently of the four plates or "discs" of secondary epiblast, and that moreover the number of these discs contributing towards the formation of the body wall, is not four but five, or even six (if we include the independent origin of the proboscidian epithelium), it need hardly be insisted that these comparisons, however ingenious (l. c., p. 279—281, 288), lose all foundation. Bergh's suggestion that the œsophagus of Lineus might arise by the coalescence of the cephalic and ventral plates (l. c., p. 281) is, moreover, negatived by my own results (see below).

primary epiblast has been disintegrated, I take it to be more probable that the greater part of it is carried off by the mesoblast cells, and plays a further part in the formation of the larva.

The differences in the mesoblast cells, which were noticed, may be partly due to the different methods of preparation; nevertheless, I will briefly describe them. Sometimes they have all a rounded shape with one distinct nucleus, strongly stained by carmine, sometimes more nuclei appear to be present, at least more elements which have the same affinity for carmine as the nucleus, and are often regularly arranged around a central nucleus. Sometimes I even noticed mesoblast cells which had all the appearance (l. c. (30), pl. ii, fig. 28) of containing faded nuclei, that had perhaps belonged to more than one cell,[1] in each of which a distinct nucleolus was visible. Other mesoblast cells contain small, strongly refractive bodies, whilst it may in conclusion be noted that the protoplasm of the mesoblast cells is not always similarly affected by picro-carmine; sometimes remaining colourless, sometimes becoming light rose-coloured. I mention these different variations because I feel sure that they may be of considerable cytological importance, although for myself I have no leisure to consider them from that point of view in the present paper.

The primary epiblast has one more function, which we have not as yet considered. It forms a paired invagination, one on each side of the blastopore, commencing as a shallow depression (l.c. (30), pl. i, figs. 35—42; pl. iii, figs. 46—48) which gradually deepens, finally closes up and is nipped off from the primary epiblast. A spherical sac with a central cavity thus appears in a certain stage of development on each side of the blastopore (Pl. XXII, figs. 4 and 5). These sacs come to lie in the blastocœl and are only later on enclosed in the secondary epiblast at the time when this increases in size and coalesces (figs. 5, 6, 11). During this process they moreover change

[1] Dr. van Rees tells me that similar "scavenger" cells were noticed by him during the process of the metamorphosis of the larva of Musca vomitoria.

their position (cf. fig. 6) and become situated no longer ventrally but laterally. When this change has been effected, their central cavity again communicates with the exterior by two channels, not this time by the side of the blastopore (mouth), but wholly lateral, viz. in the cephalic furrows. This secondary communication with the exterior is at the same time the definite one. The sacs have now become the lining of the cavity which is found in the posterior brain-lobes. These lobes in the adult are known as the "cephalic sacs," as "the side organs," etc., of the different authors. In a former publication I have attempted to demonstrate that the cavity must be subservient to a curious direct respiratory process of the hæmoglobiniferous nerve tissue.[1] Embryology now renders it probable that they may at the same time have a sensiferous significance, as was the more generally accepted, and, in a certain sense, the current hypothesis.

It is, indeed, almost impossible not to look upon the inner cavity of these (respiratory) sacs as clothed by a sensory epithelium, when we consider that this epithelium arises from the outer surface of the primary epiblast, from which in addition only the epithelium of the proboscis takes its origin. That the latter is primarily of an eminently tactile significance was already noticed by me in an earlier volume of this journal (p. 349, 1883), when comparing Graff's description of the origin of the proboscis in certain Rhabdocœls with the condition of things in the Nemertea. The view is, moreover, strengthened by the very elaborate and most copious innervation of the proboscis in all Nemertea, especially in those that are known to make the most constant use of this organ.

The facts just recorded are all the more curious since I must now emphatically state that one of the results of my investigation is this, that n o p o r t i o n o f t h e c e n t r a l n e r v o u s s y s t e m o f L i n e u s t a k e s i t s o r i g i n i n t h e e p i b l a s t either primary or secondary, but that the whole nervous system is of a mesoblastic origin. Those epithelia—in addition to that of

[1] 'Zur Vergl. Anat. und Physiol. des Nervencyst. der Nemertinen; Amst. Akad.,' 1880.

the outer surface of the body—which do arise from the epiblast, and afterwards become closely connected with the central nervous apparatus, would thus seem to be eminently sensory epithelia. It may be argued that it would indeed be much less comprehensible that a sensory epithelium should, by a secondary process, come to arise out of mesoblast cells than that the central apparatus should do so.

One word more about the posterior brain-lobes (side organs auct.) and their central cavity. Earlier investigators have come to the following conclusions concerning them. Bütschli, who examined Pilidium larvæ (16) (of another species of Nemertea than our Lineus), describes these organs as epiblastic invaginations. He moreover mentions the presence of two diverticula of the œsophagus : a phenomenon wholly divergent from what was described above. Nevertheless a similar arrangement to that described for Pilidium by Bütschli is noticed in our Lineus at a late stage of development. These anterior diverticula of the œsophagus (l. c. (30), fig. 87) could, however, be demonstrated to be in no relation whatever with the posterior brain-lobes.

Metschnikoff (14), who also studied the Pilidium larva, regards the "side organs" as œsophageal diverticula. Barrois (21) similarly gives a detailed description in his account of the development of our identical Lineus obscurus of the origin of two lateral diverticula of the œsophagus, which remain connected with it during a certain period by a special peduncle, figured in his plates, and disappearing later on, when the communication with the œsophagus is finally given up. Barrois tells us that these diverticula become the side organs of the adult.

It is a considerable difference on an important point between my own observations above recorded and those of Barrois, that this investigator, together with Metschnikoff, looks upon the œsophagus as the starting-point for these diverticula. His account of the prolonged connection between œsophagus and side organs is not in accordance with the real facts, which have taught us that the origin of the

sacs, their shifting to a more lateral situation, and their definite, though secondary connection with the (secondary) epiblast takes place in early developmental stages, and that in those stages nothing is seen (in actual sections) of any connection with the œsophagus.

The stages which I have described above as occurring later on during the development of the œsophagus of Lineus must somehow have misled even so accurate an observer as is Barrois.

b. The Hypoblast before the Shedding of the Primary Larval Integuments.

The general features of the process by which wandering mesoblast cells take their origin in the hypoblast have already been recorded above.

We must now inquire more closely into the nature and significance of a phenomenon, the interpretation of which has given me very considerable difficulties. Whereas the communication between the archenteron and the exterior, by means of a wide blastopore, is most evident in the earliest stages of development, I find without exception that in later stages (cf. fig. 7) the cavity in that portion of the intestine which commences to grow backwards is closed anteriorly, and that in front of this another portion of the embryonic intestine constantly remains in open communication with the exterior, but is never in communication with the posterior portion of the gut (figs. 8 and 9). We will provisionally call these two cavities the larval fore-gut and hind-gut. The anterior cavity or larval fore-gut opening outwards ventrally, is narrow and flattened from before backwards (cf. figs. 10 and 11). In accordance with this the outer opening is no longer a circular blastopore, but a more or less crescentic slit (l. c. (30), pl. ii, figs. 31 and 32). In the commencement I thought it probable that the original blastopore of figs. 1 to 3 would become closed, and that then the archenteron would be pushed inwards by an epiblastic ingrowth, giving origin to a distinct stomodæum, which would then be the anterior cavity above described. Two more considerations

gave probability to this hypothesis. In the first place, I was able to show that the œsophagus of the adult worm developed out of this stomodæum. Secondly, Metschnikoff describes in both his papers (14 and 25) on the development of Pilidium and Lineus, an anterior portion of the gut which entirely corresponds in situation and delineation with the cavity just described for Lineus. Metschnikoff looks upon this portion of the gut as decidedly an epiblastic stomodæum, and also notes that the œsophagus of the full grown animal developes out of it.

Further investigations carried on with very numerous larvæ of the earlier stages, obliged me to change my original interpretation just now stated. In the first place, I never found a preparation in which the blastopore became closed in loco, i. e. on the level of the epiblast. The tissue separating the larval fore- and hind-gut was always situated further inwards. It thus appeared more probable that the archenteron subdivided into two portions of which the posterior one became entirely shut off from the exterior. I indeed succeeded in finding sections (l. c. (30), pl. i, fig. 8) in which the commencement of such a separation and local narrowing was clearly seen. This preparation at the same time convinced me that the cells which will clothe the larval fore-gut are quite as evidently hypoblast cells as are those which build up the wall of the hind-gut, and that they are in no way of an epiblastic nature. This is the more important, as very soon a certain though slight amount of difference between the cells paving both cavities can be observed, since on this account a presumed epiblastic origin of one of them might seem all the more probable.

Now, if the larval fore-gut were indeed an epiblastic ingrowth we would have to picture to ourselves its increase in depth, either by a continued infolding at the mouth, or by a continued cell division, new epiblast cells being sent inwards to complete the ingrowth when once it had begun.

That no active continued infolding takes place can be easily verified by comparison of the sections which, when once the

secondary epiblast can clearly be traced, would immediately reveal such a process by respective changes in situation of parts, &c.

The second process can of course not be as emphatically denied as the first, but as the larval fore-gut always has a certain size when it originates, and was never seen directly to spring from the epiblast (*vide* supra), it is a far more acceptable view that the increase in depth of this front portion is due to repeated subdivision of the hypoblast cells forming this region, than to a similar process in epiblast cells which have not with certainty been demonstrated as constituents of this region.

At all events it is evident that anyone wishing to persist in maintaining that the larval fore-gut is indeed an epiblastic stomodæum, is obliged to acknowledge that its formation or invagination begins and is clearly appreciable even before the then inferred closure of the blastopore has commenced. This is evident from such preparations as the one referred to (l. c. (30), pl. i, fig. 8). The closure of the blastopore must then take place later on, after it has wandered higher up into the gastrula. I repeat that this explanation appears to me highly artificial. For myself I fully accept the other interpretation, viz. that the external opening, leading into the gut, even when it has become narrow and crescentic, is still the original and permanent blastopore, which later on becomes the mouth of the adult without even disappearing, and the two cavities of the two regions of the gut are then wholly equivalent portions of the archenteron; they have only become separated by an internal constriction and afterwards follow their respective destination along different lines of development.

We must now inquire into the further phases through which these two portions of the intestine have to pass before attaining their ultimate structure. The posterior portion or larval hind-gut becomes the mid- and hind-gut of the full-grown worm, i. e. that portion which is characterised by the paired cæcal diverticula and which extends unaltered down to the anus. From the anterior portion or larval fore-gut, on the contrary,

originate the œsophagus, which even in the adult animal is easily distinguished from the mid- and hind-gut, by its having no trace of lateral cæca and by the great distinctness of its coating of strong cilia. In Lineus and the other Schizonemertea it is surrounded by the widened, lacunar portion of the circulatory system.

However, we shall presently see that not the whole of the larval fore-gut is transformed into the œsophagus but only the lower part, adjoining the blastopore. We have already noticed that the larval fore-gut is characterised by a flattened appearance, the lumen being similarly narrowed, and in the upper portion often actually disappearing in the middle, and only remaining visible right and left of this median concrescence, like the two globes of a dumb-bell. This portion apparently becomes converted into the nephridial system, which is situated in the adult right and left of the œsophagus in the blood lacuna which surrounds this.

We will now rapidly trace the chief phases in the development of œsophagus and nephridial system as they were observed by me. The first traces of the definite œsophagus consist in a cell-proliferation appearing in the walls of the lower part of the larval fore-gut. The constituent cells become much smaller than they were at the commencement: the nuclear elements being thus more numerous in this lower portion it can be easily detected in stained sections by its more intense colouration. A layer of mesoblast cells may be seen to develope simultaneously and to form the enveloping tissue for the hypoblastic cellular layer, which is transformed into the cellular surface of the definite œsophagus. When a certain degree of development has been reached this œsophagus, arising from the walls of the larval fore-gut, secondarily coalesces with the cavity of the larval hind-gut, as may be gathered from a comparison of the figs. 9 and 13. Elsewhere I have given more elaborate figures of the actual sections from which the details of the process may further be gathered (l. c. (30), pl. ii, fig. 30; pl. iii, figs. 47, 48; pl. v, figs. 80, 81, and 84).

When the definite œsophagus has entered into its secondary communication with the hind-gut, the upper portion of the cavity of the larval fore-gut has become separated from the lower portion that gives rise to the œsophagus. This upper portion is, moreover, characterised in this stage by the disappearance of its central lumen. Laterally two lumina remain persistent, which are thus direct derivates of the primitive archenteron. Cell-proliferation may also be noticed around them, and from these arise the paired nephridial ducts.

When we remember that it is only in recent years that the nephridial system of the adult Nemertea has been definitely recognised, and that even now it is not always easily demonstrable in the adult, it will be understood how exceedingly difficult it is to trace this system in the early stages of which we are now treating; several times it was even impossible to detect its presence. For this reason I must congratulate myself that more than one observation has corroborated the views expressed above about the development of the nephridian system. A cellular, closed vesicle was more than once noticed, lying in the immediate vicinity of, but separated from, the œsophagus and evidently having developed one of the same mother-tissue (l. c. (30), pl. v, figs. 73—75, 82—86).

The nephridia apparently remain during a long period in a more or less embryonic phase. Oudemans (28) has demonstrated that the number of excretory pores (the secondary paired openings by which the nephridial ducts communicate with the exterior) increases as the animal increases in size, and this may further tend to prove that in Lineus the nephridial system attains its full development only late. This must partly serve to explain why I often found it so difficult—even in older larvæ—to distinguish the excretory apparatus. I nevertheless feel convinced that the phases of development of this system, as traced above, are in accordance with the actual facts, although it is especially on this head that I look forward with great avidity to further evidence. All other points in the ontogeny of Lineus recorded in this paper have been verified

over and over again: for the development of the nephridia however, I can as yet only refer to a more restricted number of observations, all nevertheless, in accordance with each other. There remains for the present a considerable blank in our observations between the vesicular stage of the nephridium and that in which we find it in the adult worm.[1]

Moreover, I must for the present leave undecided whether the whole of the cell-material of the primitive fore-gut is used up in the formation of œsophagus and nephridia, or whether a portion of it is resorbed or converted into amœboid mesoblast cells.

When the mid- and hind-gut has for the second time entered into communication with the exterior by means of the newly-formed œsophagus, its cell wall shows a very marked difference from that of the latter (cf. l. c. (30), pl. v, fig. 81, also pl. vi, fig. 65). In some preparations I find the epithelium high, in others much lower; in some there is a decided lumen of this part of the gut, in others not. I would feel inclined to accept the view that not all the original hypoblast cells pass into this epithelium but that some remain lying in the cavity of the intestine, and are there digested as embryonic pabulum. An anus is not yet present in these stages, even when the œsophagus has already coalesced with the intestine. There is a median longitudinal infolding of the intestinal epithelium along the back of the animal, in the region where the proboscidian sheath will by-and-by develope. This fold (fig. 14) is best seen in transverse sections (l. c. (30), pl. iv, figs. 64—67).

[1] It is only with the utmost reserve that I venture to point to this origin of the nephridia as paired outgrowths from part of the wall of the archenteron, as being a process which undoubtedly offers certain points of resemblance with the origin of a true enterocœl. This ontogenetic process must, however, be studied in further detail, and more should moreover be known about the nephridial cavities in the adult throughout the whole class of the Nemertea before we are justified in proclaiming homologies between these latter cavities and the enterocœl of other Metazoa. Oudemans' researches (28) do not lend any support to such homologies; rather the contrary.

c. The Mesoblast.

We have already fully considered the origin of the mesoblast cells and their characteristic properties. Only little remains to be added.

Once freely moving about in the blastocœl they very soon accumulate against the inner surface of the plates of secondary epiblast, in the commencement retaining their more massive shape (l. c. (30), pl. ii, figs, 23 and 27; pl. iv, fig. 61), but gradually flattening out and diminishing in size. Thus, at the same time the difference between epiblast and mesoblast cells becomes less and less marked. Dorsally the mesoblast cells have a very distinct tendency to unite into a separate layer of flattened cells (l. c. (30), pl. ii, figs. 29 and 30; pl. iii, fig. 50; pl. iv, fig. 68), which, of course, gives a great distinctness to the three different embryonic layers (fig. 6). The cells which are laterally added to this mesoblast layer, and thus constantly tend to extend its surface, show very instructive transitional forms between the more massive free mesoblast cells and the flattened ones composing the layer. There is a special accumulation of mesoblast cells in the prostomium, where they very soon fill up the space between the coalescing cephalic plates of secondary mesoblast, and surround the incipient proboscis, the inward growth of which has been described above (figs. 9, 11, and 12).

This accumulation of mesoblast cells is, at the same time, the first step towards the differentiation of tissues other than epithelial in the larva. We have already in the preceding pages hinted at the fact that the nervous system in Lineus is of mesoblastic origin; this must now be demonstrated. We very soon meet with a further differentiation amongst those mesoblast cells, which we find applied against the coalescing plates of secondary epiblast, i. e. against the larval integument, and which form a massive group in the prostomium, a comparatively thin cell-sheet in the rest of the body. The process of differentiation of these embryonic cells into (1) muscle-cells

and (2) nerve-cells has been followed by me in detail (l. c. (30), pl. iv, figs. 58—60, 64—71; pl. v, figs. 72—83, 87—89) in very numerous series of sections. They appear simultaneously; and whereas a muscle-cell may soon be distinguished by the section of the fibril developing out of it, the nerve-cells at a very early stage give rise to the so characteristic fibro-nervous core which (as in the adult animal) the cellular constituents are found to surround, both in the lateral longitudinal stems and in the brain-lobes. I cannot with certainty say whether this fibro-nervous core, composed of extremely attenuated separate nerve-fibres, arises by the outgrowth of fibres out of pre-existent nerve-cells, or by the transformation of primarily cellular longitudinal strands into fibres.

The very early period at which these fibres are distinctly visible, surrounded by embryonic cells, would make me incline towards the first view. I may, however, in still earlier stages, have overlooked rows of embryonic nerve-cells in the act of transition to nerve-fibres, because it is very difficult, at so early a period, to decide whether the embryroic cells are going to develope into nerve- or muscle-cells, and because this can in most cases only be answered with certainty after distinct fibres have made their appearance.

As well in the prostomial cell mass, as in the mesoblastic layer of the posterior region of the body, such fibrous cores are thus demonstrable at a very early period. In the prostomial mass they are from the very first arranged as they are in the adult, i. e. in two ventral brain masses, from which the lateral cords spring, and two dorsal ones forming the superior brain-lobes. Anteriorly the lobes coalesce right and left, and both halves are again united by an annular commissure, surrounding the proboscis and its sheath, and also observable in a very early phase (l. c. (30), pl. v, fig. 81). It is absolutely impossible, in all the numerous series of sections which I possess of these early stages, to find one single instance that might be adduced in favour of an epiblastic origin of the nervous system. It must be borne in mind that it would be the secondary epiblast in which the process of the origin of the nervous system would

have to be demonstrated; the primary having been isolated from the tissues forming the rest of the body by the formation of the secondary epiblast in the way described above, and only contributing towards the formation of the sensory epithelium of the proboscis and of the posterior brain-lobes in the way traced in preceding pages.

This fact must be remembered when we consider the apparently unexpected fact of the mesoblastic origin of the nervous system. A certain number of the mesoblast cells did arise out of the primary epiblast, and it is in no way improbable that these might in the first place contribute to the formation of the nervous system. If this could be proved it would certainly not make such a very great difference, that the nerve-cells, instead of developing into the central nervous apparatus in loco, first changed their situation in accordance with the further changes in the primary epiblast and with its final rejection. The nervous plexus, which some years ago was demonstrated by me as being present just outside the layer of circular muscles, can be observed in early larval phases; in very young animals, only a few millimetres in length, it is indubitably present, and as the tissue composing it passes in the most gradual manner into that of the lateral nerve stems, I have no doubt that it developes in exactly the same way and at the same period, i. e. from the mesoblast cells just mentioned. The same must be admitted for the few separate and independent nerves, that have been observed in Lineus (as in other Schizonemertea) to emerge from the central apparatus, viz. the nerves to the tip of the snout, the nerves for the proboscis, and the so-called vagus nerve which springs from the lower brain-lobes and innervates the œsophagus. The mediodorsal nerve (so called proboscidian-sheath nerve) is no more than a local thickening in the cylindrical plexus just alluded to. In tracing the origin of the musculature of the proboscis we shall see that the development of its nerves in the way just indicated is not only probable and intelligible, but that this view may be said to be the only one that fits in naturally with the development of its musculature.

Before passing from the nerve tissue to the muscular, mention must be made of the ulterior phases of the two epiblastic invaginations, which we noted in an earlier stage and about which we remarked that they would develope into the central cavity of the posterior brain-lobes. These ulterior phases have also already been described above, and we have only to add that the development of nerve-cells out of mesoblast cells which very soon surround the spherical sacs when they come to lie in the blastocœl (l. c. (30), pl. ii, figs. 39 and 40; pl. v, figs. 74—80, 87), leads to the ultimate coalescence of the posterior with the anterior and superior lobes, and that in this phase hardly anything would denote the independent development and ulterior coalescence of the component parts.[1] The question whether the original epiblast cells, coating the interior cavity, indeed produce nothing but the epithelial lining of this cavity in the adult, or whether they might also contribute towards the formation of the nerve-cells by which this cavity is immediately enclosed, will always remain very difficult to answer with absolute certainty. For my own part, it will be obvious from what I have remarked about the development of the nervous system in the mesoblast, that I should very much hesitate in accepting the latter view, that, on the contrary, I expect to find the nervous tissue which has a specific sensory nature—the epithelial lining of the cavity—and is epiblastic in origin, to be essentially distinct from that to which a conductive and perceptive significance must be accorded, the latter being of mesoblastic origin.

Another component part of these posterior brain-lobes, viz. the accumulation of spherical refractive cells in their posterior portion, which has more than once been described in the adult (17, 20), was seen by me to develope in the tissue when it was already distinctly composed of nerve-cells and fibres. Certain

[1] Attention should here be drawn to a certain resemblance in the origin of these structures in the Nemertea and the origin of part of the brain-lobes in Mollusca (Dentalium, Pteropods) and Polyzoa as tubiform or vesicular invaginations of the epiblast according to the researches of Kowalevsky, Harmer, and Fol.

cells of the lobes then assume this particular appearance, and no derivation from œsophageal tissue, which I held to be not improbable in a former publication when I could only consider the embryological data as they were furnished by previous authors, can be any longer upheld.

Of all authors who have given descriptions of the origin of the nervous system in Nemertea—and from all of whom my own results differ—few give such full details as Salensky in his latest article (27). Although he has examined a different species, belonging to a different group of Nemertea, in which development is direct and no Desor's larva occurs, still I may be allowed to presume that on this head the observations of the Russian naturalist are less accurate than others we owe to his trained eye. In the Dutch version of my researches I have given a full account of the points of divergence between his results and mine, and have attempted to show that the figures which he gives, fit in much more naturally with my interpretation of the facts as I found them in Lineus than with his own, and make it highly probable that also in Amphiporus viviparus the nervous system has a mesoblastic origin. In that case we may at the same time leave Salensky's suggestion—which, however, he has far from proved—viz. that the lateral nerve-cords arise as posterior outgrowths from the brain-lobes, out of further discussion, referring to the cited memoir.

Further products of the mesoblast besides the nervous system are the muscular layers of the body wall, those of the proboscis and the proboscidian sheath, and the hyaline ground substance in which all these are embedded, and which wholly fills up the space between the intestinal, nephridial, generative and vascular cavities. I will now give a short account of the further development of these mesoblastic products, together with which will be discussed the origin of the cavities of the proboscidian sheath and of the blood vascular system.

After the formation of the five plates of secondary epiblast, which are to furnish the epiderm of the adult, has commenced in the way above described, we saw that the mesoblast cells

very soon begin to accumulate against the internal surface (figs. 5, 6, and 11). I expect that it is this accumulation which has also been observed by Barrois, and which has been very differently interpreted by him, viz. as a proliferation of the plates of secondary epiblast from which the mesoblast originated. Our actual sections have, however, sufficiently demonstrated that it is not a proliferation but an accumulation of mesoblast cells which was observed, and that the latter originate in a different way, not from the secondary but from the primary epiblast.

It can without difficulty be demonstrated that the outer layer of longitudinal fibres developes at an early stage out of the mesoblastic material accumulated against the secondary epiblast, and that the latter remains a unicellular layer for a comparatively long period, long after the larva has been set free. The appearance of the outer longitudinal muscular layer is, as we have already noticed above, simultaneous with that of the longitudinal nerve-stems, as is that of the brain-lobes with that of the muscular tissue in the head. The details of the transformation of embryonic into muscle-cells have been figured elsewhere (l. c. (30), pl. iv, figs. 64—71).

It is only much later that the circular muscular layer and the inner longitudinal layer make their appearance, so as to be clearly distinguishable (l. c. (30), figs. 69, 70). The larva has then long ago shed the covering of primary epiblast, and moves about in the gelatinous strings by which the egg-capsules were enclosed when they were deposited by the animal, and which now appears to serve as a pabulum for the young larvæ. That the development of these two muscular layers is indeed of comparatively late occurrence can be more especially demonstrated in the dorso-median region, where there is present a longitudinal fold in the hypoblast (fig. 14). The space enclosed between this fold and the developing body wall is nothing else than the original blastocœl, and in this dorso-median region must arise, in addition to the muscular layer just mentioned, the proboscidian sheath with its internal epithelial covering and its external musculature. Both of these are also compara-

tively late in making their appearance, and the proboscis of which we noticed the first origin has long penetrated into the blastocœl, has even become attached by embryonic muscle-cells (figs. 9 and 13) to the developing muscular body wall before its sheath has become a distinct layer. And even when this sheath has appeared it is first only one cell layer thick (figs. 12—14), and shuts off a cavity round the proboscis from the rest of the blastocœl. Before this, however, the muscular coat of the proboscis itself has come into existence. We have seen that the inner cellular lining of the proboscis is directly derived from the primitive epiblast. The further developmental phases of this epithelium have been repeatedly observed by myself, and at the same time the process by which the muscular coats that ensheath this internal epithelium, and that give to the organ its peculiar mobility and retractility, gradually develope out of mesoblast cells that apply themselves against the epiblastic invagination as soon as it grows backwards into the blastocœl to form the first trace of the proboscis. Elsewhere this process has been more elaborately figured (l. c. (30), pl. iv, figs. 58 and 59, 66; pl. v, fig. 81); it is diagrammatically represented in figs. 9, 11, and 13. These figures may at the same time elucidate how the development of the nervous tissue in the proboscis goes hand in hand with that in the head, and continually remains in connection with the central lobe, whatever further differentiation may go on in these particular mesoblast-cell groups, out of which both the central nervous system and the muscles and nerves of the proboscis take their origin.

The different facts here mentioned about the growth of proboscis and proboscidian sheath are first of all observed in the prostomial region, and here the unicellular wall of the embryonic proboscidian sheath fuses with the developing musculature of the head just in front of the brain-lobes. However, it is then not yet present in the metastomial region, where the muscular extremity of the proboscis was already noticed by us as fusing with the muscular body wall (fig. 13), before the sheath has appeared. This happens nearly simultaneously with

the appearance of circular and inner longitudinal muscular layers, but in the beginning no muscular elements are detectable in the sheath, the cellular epithelium being the only representative of it, especially in the head. Only gradually muscular fibres develope outside of this epithelial lining, and these fibres again differentiate into different layers. There is thus absolutely no escape from the conclusion that the epithelial lining of the proboscidian sheath arises out of a laminar arrangement of mesoblast cells. So do the muscles of sheath and proboscis, as well as the free corpuscles floating in the fluid which is found between the proboscis and its sheath, and is of primary importance in the act of expulsion of the proboscis.[1]

We must now return to the cavity in which the proboscis moves. In the early stages this is no other than the free cavity between the body wall and the hypoblast, which has not arisen—as the numerous preparations clearly show—by a splitting of the mesoblast (schizocœl), nor by a differentiation

[1] It must here be observed that the results which I have obtained concerning the development of the proboscidian sheath apparently give no support to the hypothesis which I ventured to make a few years ago ('Quart. Journ. Micr. Sci.,' vol. xxiii, 1883, p. 349), according to which if we regard the hypophysis of Vertebrates as a rudimentary proboscis of their Invertebrate ancestors, we might also compare the notochord to the proboscidian sheath. Amphioxus, developing its notochord out of a dorso-median furrow of the hypoblast, it would have been a most valuable argument for this hypothesis if also in Nemertea the proboscidian sheath arose in a similar way. This we have seen is not the case. We must, however, not forget that if any such positive argument is not furnished by the facts of ontogeny, neither is any argument contrary to that hypothesis implied in those facts. For if we remember that the dorso-median proboscidian sheath arises out of mesoblast cells, and that the mesoblast in part developes out of the hypoblast, it would not be impossible that, by the help of some new method of investigation which would allow us to follow the origin of the individual mesoblast cells, we might after all demonstrate that the mesoblast cells that become the proboscidian sheath are all hypoblastic and originate out of this primary layer in the regions where they are subsequently found, i.e. medio-dorsally. And in that case, which is in no way rendered improbable by these researches, the comparison between notochord and proboscidian sheath would receive very emphatic support.

from the archenteron (enterocœl), but is nothing else than the original segmentation cavity, since it can be directly traced back as far as the blastula of figs. 1—3. This cavity is generally called—at least in the early stages—the blastocœl. I think it will be advisable and will prevent misunderstanding to limit the use of that name to the very earliest developmental stages, and to give to such cavities in the body of the larva and of the adult as can be demonstrated directly to arise out of this segmentation cavity a separate name, for which I would propose that of archicœl. It indicates that such a cavity has indeed a very primitive significance, and must be held distinct from the archenteron and its derivatives.[1]

The amœboid mesoblast cells, budded off both from epi- and from hypoblast thus are set free in the archicœl; whilst they here accumulate in different regions and develope into different tissues, they do not fill up the whole of the cavity; what remains of it may serve for different purposes, but in no case should its significance as part of the primary archicœl be overlooked. In the adult Nemertea it is the cavity of the proboscidian sheath, and, as we shall demonstrate further on, also the cavity of the blood-vascular system which must be regarded as an unmistakeable Archicœlom.

Now, it may, perhaps, not always be an easy task to distinguish an archicœlic cavity from a schizocœlic, especially in cases where the developing tissues bulge out and mask the archicœlic cavity temporarily from our view, so that this cavity only reappears later on, and is then liable to be looked upon as appearing for the first time as an effect of a special cause. We must well keep in mind that these two phenomena are essentially different, and that the continuity of the original segmentation cavity, whether temporarily invisible or not, is a passive phenomenon, for which no further adaptative and hereditary processes have to be invoked, whereas the appearance of a cavity by actual

[1] Claus ('Typenlehre,' 1874) und Hatschek ('Entwgesch. der Anneliden,' 1878) have already called attention to the significance of this kind of primary body cavity. Hatschek even distinguishes a group of Vermes archicœlomata (l. c.).

and active splitting of the mesoblast requires the demonstration of anterior phases in which no such cavity was present, of the successive steps by which it gradually arose, and of the special adaptative significance of these transitory stages in each case.

That the latter process is far more complicated and in many cases more unintelligible need hardly be insisted upon. Moreover, the term "schizocœl," introduced by Huxley, originally had a much more definite and limited meaning than it has nowadays gradually acquired. Huxley's own words are ('Quart. Journ. of Micr. Sc.,' vol. xv, p. 54): "In the Schizocœla a perivisceral cavity is formed by the splitting of the mesoblast;" and the same author is not unwilling to look upon the perivisceral cavity of the Polyzoa as "a blastocœle, more or less modified by the development of the mesoderm" ('Anatomy of Invertebrated Animals,' p. 460).

O. and R. Hertwig, in their contributions to our appreciation of the nature of the different forms of cœlom, have applied the term schizocœl to the perivisceral cavity of many invertebrate animals, and there is a strong tendency to apply that name to all such cavities that arise in what they call the "mesenchyma." And when they come to ask, "Wie verhält sich das Schizocoel der Mollusken zu dem Blastocoel ihrer Larven? ('Coelomtheorie,' p. 13) they immediately answer: "Von Anfang an ist ein weites Blastocoel vorhanden, dessen Raum durch die zunehmende gewebebildung eingeschränkt wird. Die überbleibenden spalten sind die ersten Anlagen des Schizocoels, dass sich nun secundär wieder zu einem einheitlichen Raum gestaltet. Zwischen Blastocoel und Schizocoel würde sich demnach eine ununterbrochene Continuität nachweisen lassen."

The question, as we have formulated it above, whether the origin of this cavity must be looked upon as a passive phenomenon or as an active excision, originally adaptative and rendered permanent by heredity and selection, is thus in silence passed over by them. I have sufficiently insisted upon my reasons for keeping these two processes well apart; and it will

then be better understood why I must emphatically warn the reader against this tendency to give such considerable latitude to Huxley's term of schizocœl. This is all the more necessary as it is well known that in the Vertebrates and in certain higher Invertebrates an active splitting of the mesoblast may give rise to a true enterocœl. If we adhere strictly to Huxley's terminology we would here be obliged to apply the name schizocœl, and Huxley himself is naturally led to ask the question (l. c., p. 56): "whether the splitting of the mesoblast in the Vertebrate may not have a different meaning from the apparently similar process in the Arthropoda, Annelida, and Mollusca?" This question, which everyone will answer in the affirmative, so far as some of these groups are concerned, is the best proof that the name schizocœl was not a fortunate one. The Hertwigs, instead of suppressing it, have considerably extended its significance in a way which I consider to be most unadvisable, and hence I propose to apply the name archicœl when it can be unquestionably demonstrated, as in Lineus, that the cavity has indeed been present from the very beginning, and to reserve the name of schizocœl for those cases when it can similarly be demonstrated that the perivisceral cavity originates by a process of active scission, and when this scission can in no way be looked upon as a derivation, either of archi- or of enterocœl. Bütschli ('Morph.-Jahrb.,' vol. viii, p. 474) has already attempted to bring about a comparison between our archicœlom and the blood-vascular cavity of the Vertebrates. I need not point out that what we have found to exist in Nemertea appears to lend considerable support to this hypothesis.

Before concluding our remarks about the origin of the different layers of the body wall we must not omit to notice the presence, in addition to the muscle-cells of the very characteristic connective tissue that is found between the muscular fibres, between these and the cellular epiderm, and between these and the wall of the intestine. I need not point out that this connective tissue is eminently of mesoblastic origin, nor repeat that the space between the muscular body wall and the

intestine is wholly filled up by the connective tissue, which there obtains a hyaline, transparent, and gelatinous character, with cells sparsely interstrewn, and with variously-shaped fibres.

The spaces belonging to the blood-vascular system of Lineus, viz. the lacunæ round the œsophagus and in the head, and the three longitudinal vessels with metameric anastomoses in the rest of the body, arise merely by the fact of the connective tissue not obliterating these spaces mentioned, but there affecting the shape (1) of an endothelium lining the cavities, (2) of a basal membrane, (3) of an outer coating in which separate fibres may sometimes be distinguished. The direct passage of the archicœlom into the blood-space can be very demonstratively studied in the œsophageal region, and also for the dorsal vessel beneath the proboscidian sheath (fig. 14). Elsewhere this has been figured more in detail (l. c. (30), pl. iv, figs. 64—67; pl. v, figs. 72—83 and 87).

It may here be added that the three longitudinal vessels cannot be easily observed during all the early larval stages, principally because of the close application of the hypoblast against the body wall.

However, when we have only the choice between two possibilities: (1) that the blood-vascular system, together with the lacuna round the œsophagus and the blood-vessel inside the proboscidian sheath, arise by a splitting process *ad hoc* in the mesoblast; or (2) that, together with the proboscidian cavity, inside which the dorsal vessel is partially enclosed, it represents the last remnants of the archicœl, i. e. of that cavity in which already in the blastula stage a fluid was contained, and a movement of this fluid was possible, there can be no doubt which of the two is the more simple and more natural explanation. The blood-vascular apparatus as well as the proboscidian sheath are thus of very primitive significance. A fact of some importance is this, that in the adult the posterior brain-lobes are bathed by the circumœsophageal blood-lacuna. They accordingly remain situated, notwithstanding their coalescence with the anterior brain-lobes and their external openings,

in the cavity where we saw them originate—the archicœl (l. c. (30), pl. v, figs. 75—79). This explanation is again far more natural than another, which would attribute the relative situation of brain-lobes and blood lacuna to a later development of the blood system by which it came to surround these lobes.

A third possibility, viz. that the blood system of these animals has arisen by a process of hollowing out of solid cell-rows and cell-groups, must be emphatically rejected on the authority of numerous preparations.

In the midst of the outer layer of longitudinal muscle-fibres the connective tissue affects another important character. It here remains visible as a continuous cylindrical sheath separating an outer from an inner layer of these fibres (l. c. (30), pl. v, figs. 88 and 89), and distantly reminds one of the layer of cambium tissue in dicotyledonous plants. I mention this name because I feel assured that the increase in thickness of this muscular layer is for the greater part due to a gradual passage of cells from this more neutral layer into longitudinal muscle-fibres. The mode of origin of the first muscle-fibres in the embryo would be thus continued in the older larvæ, for it must be remarked that the arrangement just mentioned can only be observed in young animals that have already attained a few millimetres in length, and has again disappeared in the adults.

With respect to the circular and internal longitudinal muscular layers, which have been already described as appearing later than the outer longitudinal muscles, it must still be noted that they only reach as far forwards as the posterior brain-lobes. The circular layer disappears in the fold between the posterior and anterior upper brain-lobes. It is noteworthy that this spot corresponds to the region of coalescence between the cephalic and the ventral plates, which at the same time may be said to mark off a prostomial from a metastomial region. Barrois has already called attention to this phenomenon.

We have now finished discussing the different derivatives of

the mesoblast, and have only to add a few words concerning the further development of the cellular layers of the epiderm. The outer layer, i. e. the secondary epiblast, retains its character as an embryonic layer for a very long time, although in a very early stage vacuolation may be noticed in a large majority of these cells (l. c. (30), pl. iv, figs. 63, 64), followed by a change in their elements into distinct, flask-shaped unicellular glands, such as are so copiously present in the epiderm of the adult. The remaining cells carry the numerous cilia, which in the very early stages can be distinctly noted to be separated from the body of the cells by a most distinct transparent cuticula. Whether the deeper glandular layer, which is also very characteristic in the adult, also arises out of this same layer of secondary epiblast, could not be ascertained beyond all doubt; it appears, however, very probable.

Another derivative of the epiblast are the generative sacs, although with respect to these I cannot speak with absolute certainty. I find the embryonic generative sacs—right and left and intercœcal—connected with the epiblast by a bridge of tissue which cannot be the earliest condition of the ejaculatory ducts because those outer openings and their ducts lie dorsally of the lateral nerve stems, whilst on the contrary, these embryonic connections are found below the nerve stems (l. c. (30), pl. v, figs. 88 and 89). Moreover, the connections disappear in older stages and the definite ducts and openings must undoubtedly arise de novo. However, I have no preparations of the very earliest stages of the development of the generative sacs, so that as yet I can only invoke great probability, not certitude, about their actual origin out of the epiblast.

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EXPLANATION OF PLATE XXII,

Illustrating Professor A. A. W. Hubrecht's "Contributions to the Embryology of the Nemertea."

Diagrammatic sections through larvæ of Lineus at different stages. The primary epiblast is indicated by a light yellow, the secondary epiblast by a darker brown, the hypoblast by a pink, the mesoblast by a grey colour. Figs. 1—6 and 14 and 15 are transverse sections, perpendicular to the longitudinal axis; Figs. 7—9 and 13 are longitudinal and parallel to this axis; Figs. 10—12 horizontal.

Fig. 1.—The gastrula stage.

Fig. 2.—First appearance of the mesoblast, and of the secondary epiblast.

Fig. 3.—The secondary epiblast is gradually overcapped; the dorsal portion of it arises by delamination.

Fig. 4.—Two epiblastic invaginations (the cavities of the posterior brain-lobes or "cephalic sacs") appear, right and left of the blastopore.

Fig. 5.—The same invaginations after they have been nipped off; the secondary epiblast has increased; the mesoblast commences to accumulate against it; the blastopore is now slit-like.

Fig. 6.—The incipient cavity of the posterior brain-lobes has shifted in position; the mesoblast has acquired a more definite arrangement; in the hypoblast both portions are represented as covering each other; in the lower part of the anterior portion the incipient formation of the œsophageal epithelium is represented.

Fig. 7.—Corresponds to a stage between that of Figs. 2 and 3. First appearance of the proboscis. Subdivision of the archenteron.

Fig. 8.—Corresponds to Fig. 5, but is taken parallel to but not coincident with the median plane. Thus the cephalic and ventral plates of the secondary epiblast are included in the section, and the incipient proboscis is not.

Fig. 9.—Corresponds to Fig. 6. Further development of mesoblast round the proboscidian epithelium beneath the secondary epiblast and against the œsophagus, which is being formed out of the lower division of the wall of the anterior portion of the archenteron. The outer layer of the Desor larva ready to be stripped.

Fig. 10.—Corresponds to Figs. 5 and 8. The proboscis epithelium is on the point of coalescing with the cephalic plates of the secondary epiblast. The flattened lumen of the anterior portion of the archenteron is indicated in this figure.

Fig. 11.—Corresponds to Figs. 6 and 9. Participation of the mesoblast in the formation of brain and proboscis. The cavities of the posterior brain-lobes situated at the point of junction between the cephalic and ventral plates.

Fig. 12.—A young larva that has stripped the coating of primary epiblast. Further development of proboscis, proboscidian sheath, and mesoblast in the head (brain-lobes).

Fig. 13.—Corresponds to Fig. 12. Posterior attachment of proboscidian mesoblast. Cavity of proboscidian sheath and blood-course around the œsophageal part of the archicœlom. Coalescence of definite œsophagus with mid-gut.

Fig. 14.—Corresponds to Figs. 12 and 13. First appearance of the lateral nerve stems in the mesoblast.

Fig. 15.—Adult stage, transversely cut immediately behind the œsophageal region. The two lateral nerves, the three longitudinal blood-vessels, and the posterior parts of the nephridial char ls; the latter, as well as the intestine, rose-coloured.

Reference for combining these s into seven successive stages of development to which they severally (*T.* stands for transverse, *L.* for longitudinal, *H.* for horizontal sectio

Stage I.—Fig. 1 (*T.*). Stage II.— . Stage III.—Figs. 3 (*T.*), 4 (*T.*), 7 (*L.*). Stage IV.—Figs. 5 (*T.*), 8 (*L.*), 10 (*H.*). Stage V.—Figs. 6 (*T.*), 9 (*L.*), 11 (*H.*). Stage VI.—Figs. 12 (*T.*), 13 (*L.*), 14 (*H.*). Stage VII.—Fig. 15 (*T.*).

The Early Development of Julus Terrestris.[1]

By

F. G. Heathcote, M.A.,
Trinity College, Cambridge.

With Plates XXIII & XXIV.

My investigations, the results of which are contained in the following paper, were begun in June, 1882. I collected a number of Chilognatha and kept them in glass jars, the bottoms of which were covered with damp earth. I soon found that Julus terrestris[2] was the species best suited for my purpose, as though the eggs presented some difficulties not present in the eggs of other species, yet they were of a convenient size and were easily to be procured in great numbers.

I fed the animals on sliced apples and occasionally on green leaves, and this diet seemed to suit them well, for I never failed to get several clumps of eggs in the breeding season, though it is only this summer that I succeeded in getting them in any number. The breeding season of these animals lasts from the end of May till the end of August, though the weather has a considerable influence on the time when they begin and leave off breeding. I have observed copulation, which takes place exactly as described by Cuvier ('Régne animal,' 3rd edit., 1836, vol. ii, p. 330). I was unable to determine how long a time elapses after copulation before the eggs are laid, but believe it to be short. About four days before laying her eggs the

[1] The numbers in brackets in the text refer to the list of papers at the end.

[2] The species was kindly identified for me by Mr. T. D. Gibson Carmichael, F.L.S., as Julus terrestris, Leach.

female constructs a sort of globular case of mud for them. The bottom of this was, in the case of my animals, formed by the bottom of the glass, while at the top was a small round hole which was closed up after the eggs were laid. Four days after the case was begun the eggs were laid, each case containing a clump of about a hundred eggs fastened together by a sticky substance. By breaking away the top of the case I was able to take out as many eggs as I wanted for examination, and covering the remainder carefully with earth they proceeded with their development without injury, though if exposed to the air for about a quarter of an hour they shrivelled and were destroyed.

Methods.

The principal difficulties with which I had to contend in the preparation of the ova were, in the first place, the hard chitinous chorion, and, secondly, the great amount of food-yolk.

With regard to the first of these difficulties, I tried to remove the chorion by Bobretski's method, but I failed completely in this. I also tried to burst the chorion by endosmosis of various fluids. Perenny's fluid burst the chorion quickly, but as soon as the shell was burst in one place the contents rushed out, destroying the embryo. The state of preservation of the tissues so preserved was not satisfactory, nevertheless I gained some valuable series in this manner. I also tried various strengths of nitric acid with unsatisfactory results. I was therefore obliged to cut the ova with the chorion still on, soaking them thoroughly in the hardest paraffine and cutting rather thick sections. With regard to the preservation of the tissues I tried a great variety of fluids and also the method of preserving by heat described by Mr. Patten in his paper (12) on the development of Phriganids; but I found that I got the best results from corrosive sublimate, osmic acid, and picric acid. The last of these fluids, in some cases, burst the shell after the contents were hardened and thus enabled me to gain excellent series of sections.

The staining of my sections was a matter of much difficulty.

Borax carmine stained well in the earlier stages, while the ovum was still in the ovary, and also in later stages, when the embryo was far advanced in development; but in the intermediate stages, between about the tenth day and hatching, was wholly useless; staining the yolk-spherules equally with the nuclei. Hæmatoxylin was better, staining the nuclei deeply; but it also stained the smaller yolk-spherules so as to make it a difficult matter in some cases to distinguish between them and the nucleoli. The best fluid was alum-carmine prepared after Grenacher's method. This fluid has the advantage of staining the nuclei and nucleoli with a different tinge to that of the yolk-spherules, and the result was most satisfactory. The difficulties in the way of observing the course of development were many and were only overcome by cutting a great number of sections, only about one series in twenty being perfectly satisfactory.

The warmth of the weather had a great influence on the rate of development; one clump of eggs, for instance, was hatched on the twelfth day after being laid, while another was not hatched till the twenty-fifth. As the shorter period seemed to be the most usual, I worked out a clump of eggs which hatched on the twelfth day, and preserved a number each day, using the results as a standard by which to estimate the progress of development in other ova.

I propose in the present paper to begin with the ovum in the ovary after it has attained a fair size and to trace its development up to the time of hatching, leaving for a future paper its further development to the adult animal.

The Ovarian Ovum.

The ovum within the ovary is surrounded with a follicular envelope derived from the cells of the ovary. It has a large nucleus and a single large nucleolus, within which it is usually possible to make out two or three vesicular spaces. The body of the ovum stains slightly. The nucleus is large and distinct, stains slightly, and when viewed under a high power ($\frac{1}{16}$ oil

immersion by Reichert) consists of a network of protoplasm, chromatin granules, and more fluid protoplasm.

The nucleolus is round, very distinct, and stains very deeply. At a slightly later stage a deeply stained mass appears in the body of the ovum; this is possibly equivalent to the yolk-nucleus described by Carus in Spiders (4). It increases and finally forms a very distinct ring within the body of the ovum, as shown in fig. 1, *r*. It is a semi-fluid mass which stains deeply but does not show any structure. I have not observed any appearances like those described by Balbiani in his account of the yolk-nucleus of Geophilus (10). This mass of deeply staining, structureless material is the first food-yolk formed in the course of development of the ovum. As the latter increases in size, the ring of deeply staining material breaks up and becomes more equally distributed throughout the ovum in the form of small globules, which are more deeply stained than the rest of the cell-substance, though not so deeply as the ring before mentioned. These globules increase in size and gradually take the appearance of yolk-spherules, such as are present in all subsequent stages up to a very late period of development. Yolk-spherules continue to be formed in the protoplasm of the ovum up to a considerably later stage; such spherules invariably stain deeply while quite small, though the large spheres stain but slightly. I do not consider that the process of formation of the first food-yolk differs in any essential from that of the formation of the yolk-spherules at a later stage. The fully developed ovum within the ovary is shown in fig. 2; it is of an oval form with a thick milk-white shell, which is formed from the follicular envelope of the earlier stages. The body of the ovum consists of a great number of yolk-spherules, which are embedded in and separated from one another by strands of protoplasm which constitute a network extending throughout the ovum. At the periphery is situate the nucleus in which is a single large, deeply staining nucleolus. Examination with a high power lens ($\frac{1}{15}$ oil immersion, Reichert) shows the nucleus to consist of a network of solid protoplasm, enclosing a more fluid protoplasm in its meshes, and of chro-

matin granules which are present in small numbers (fig. 15). Within the deeply staining nucleolus, several vesicular spaces are present. I am unfortunately unable to read Russian, but from an examination of the figures of a Russian paper by Repiakoff, published in 1883, on the development of Geophilus, I imagine that the ovum of Geophilus at this stage is of similar structure.

I have been unable to observe anything of the impregnation of the ova, which probably takes place immediately before deposition.

My earliest stages occur late on the same day on which the ova are laid; sections through such ova show (fig. 3) that the protoplasmic network and yolk-spherules remain as before, but the nucleus is no longer at the periphery, but is situated in a mass of protoplasm in the centre of the ovum. This mass of protoplasm is of irregular shape, but its long axis corresponds with that of the ovum. From it amœba-like processes radiate in all directions, forming a protoplasmic network throughout the egg (fig. 17, *a*, *b*). The nucleus is no longer a distinct vesicle, but its position is marked by the chromatin granules alone. There is no nucleolus.

Early on the second day the nucleus and the central mass of protoplasm divide into two parts. The division of the protoplasm is not, however, complete, the two resulting masses with their nuclei remaining connected by a network of protoplasm. This is shown in figs. 4 and 16. The two first segmentation masses separate till they are some distance apart, though still connected by strands of protoplasm; they then divide, so that we now have four segments all connected together. This process is carried on until there are a considerable number of these segmentation masses present, and early on the third day the first formation of the blastoderm begins. At the close of segmentation the ovum consists of a number of these segmentation masses, resulting from the division of the original central mass of protoplasm. Each of these masses has a dense central portion, in which is situate the nucleus, while the outer portion is broken up into innumerable processes, which connect the masses together and permeate the yolk in every direction.

In fig. 17, *a*, *b*, I have shown the protoplasmic network under a high power. Early on the third day some of the segmentation masses make their appearance on the outside of the ovum at different parts, and there undergo rapid division, the resulting cells spreading out to form the blastoderm in a manner very similar to that which takes place in Amphipods (14). In figs. 6, 18, I have shown this process taking place.

The large flat-shaped cells which form the first beginning of the blastoderm differ considerably from the segmentation masses from which they originate. Their outline is clear and distinctly marked; their nucleus is very distinct, of an oval shape, with its long axis pointing in the direction of the long axis of the cell. A section through an ovum in this stage, when seen through a low power, shows the blastoderm cells as flat, pavement-like cells, with a long-shaped nucleus. An oil immersion lens, however, shows further details. Each cell is directly continuous with the neighbouring blastoderm cells, and also with the cells which remain in the yolk, by means of fine processes of protoplasm. There is also a difference observable in the cells within the yolk, which at this stage constitute the endoderm. Their outline is far more distinct; their nucleus is round, deeply stained, and rather smaller than at an earlier stage.

Fig. 6 shows a single segmentation mass appearing at the surface of the ovum, and about to divide to give rise to blastoderm cells.

Fig. 18 is part of a transverse section through an ovum at a slightly later stage seen, under a high power; it shows a segmentation mass which has divided, giving rise to several blastoderm cells, while some of the cells arising from the original segmentation mass remain behind in the yolk as endoderm, but are still connected with the blastoderm cells by processes.

At the stage represented in the last-mentioned section the blastoderm is present in isolated patches on the surface of the ovum. At the close of the blastoderm formation, then, the ovum consists of an external layer of flat cells—the ectoderm—with deeply stained nuclei, these cells being continuous on the

one hand with one another, and on the other with the cells in the interior of the yolk by means of fine processes of protoplasm. The cells in the interior of the yolk are the direct descendants of the first segmentation masses. They constitute the endoderm. Their fate is various. Some of them are employed in the formation of the keel, which I am about to describe in the next section; that is, in the formation of the splanchnic and somatic layers of the mesoderm. Another part is employed in the formation of the endodermal lining of the mesenteron, while a third part remains in the yolk after the mesenteron is formed, and gives rise to mesoderm cells, which are employed in the formation of various muscles and of the circulatory system. These cells will be mentioned again in the last part of this paper.

The flat surface cells enclosing the yolk constitute, as already stated, the ectoderm, and give rise to the usual ectodermal derivatives.

With regard to the retention of the primitive connection of the cells of the ovum until this stage, nothing of the sort has, I believe, been described before, except by Sedgwick in Peripatus (17). The most important part is, it seems to me, not the connection of cell to cell, but the connection of layer to layer by means of processes of the cells.[1]

Formation of the Mesoderm.

About the middle of the fourth day several of the stellate endoderm cells approach the ectoderm, in the middle line of what will eventually be the ventral surface of the embryo. Such cells are shown in figs. 7, 8, 19. Fig. 7 is an earlier stage than that shown in fig. 8. That the cells are really endodermal, and are not divided off from the ectoderm, is, I think, conclusively proved by the shape of the cells which at this period compose the ectoderm. They are flat and thin and the nucleus is long and oval, and lies in the direction of the long axis of the cell. I cannot believe that they would divide in the direc-

[1] See the figure of the morula of Limnæus, Pl. XXIV, fig. 7, of vol. xvi, N.S., of this Journal. (Ed.)

tion of their long axis; and, in fact, before they do begin to take part in the formation of the mesodermic keel, they undergo an alteration, which I shall describe. When first the endoderm cells just mentioned begin to come together in the middle line near the ectoderm their appearance is somewhat peculiar; their nucleus is small, round, and deeply stained; their form is stellate and their outline very distinct.

Processes pass from them to the ectoderm cells. This is shown in fig. 19, which is a transverse section through an ovum on the fourth day, taken in a plane such as to cut through the first beginning of the keel. When a fair number of these cells are assembled in the middle ventral line a change takes place in the cells of the ectoderm just outside them. The latter become more rounded, while their nuclei, instead of being long and oval, become round. In fact they undergo an alteration which causes them to resemble the cells which I have described as assembling immediately below them. This alteration is shown in figs. 19 and 20, which are transverse sections through the first beginning of the keel.

The ectoderm cells in the middle line, after altering their shape as I have described, increase by division, and take a considerable share in the formation of the keel. The cells in the middle line, both ectoderm and endoderm, continue to increase, and are joined by more cells from the hypoderm, and eventually on the fifth day we find a keel in the middle ventral line, something like that described by Balfour in his paper on the development of Agelena labyrinthica (16). Both ectoderm and endoderm have taken part in the formation of the keel.

When the keel is fully formed the cells of which it is composed are large, somewhat irregular in shape, and have a large nucleus. They are all directly connected together, though, owing to their being closely packed together, it is difficult to see anything of their connections, except where one cell has been somewhat separated from the others. The keel is of considerable thickness, being about six or more cells deep in its thickest part.

The keel is shown in transverse section in fig. 9 *a*, and fig. 20. At the end of the sixth day the keel is still present but an alteration is taking place in the cells of which it is composed. They are no longer round and thick, but are becoming elongated in the direction parallel to the surface. At the same time they continue to multiply and spread themselves out, so as to form two definite layers within the ectoderm (fig. 10). These are the splanchnic and somatic layers of the mesoderm. The cells of the ectoderm and of the somatic mesoderm are still connected, and also the cells of the splanchnic and somatic mesoderm.

On the eighth day the mesoderm extends round a great part of the embryo—rather more than half way round. The keel has almost disappeared (fig. 11).

On the ventral surface the cells are no longer flat but have assumed a columnar form. Their nuclei are now oval in shape, their long axis pointing, as does that of the cells to which they belong, towards the interior of the ovum. This is in fact the first formation of the ventral plate and is shown in fig. 10. While these changes are going on the remnants of the keel are disappearing. The mesoderm now becomes thicker on each side of the ventral line. This is shown in fig. 21. Both layers are concerned in this thickening, and at these points the two layers become indistinguishable. Outside the thickenings, that is, farther away from the middle ventral line, the two layers are closely applied to each other and to the epiblast as before. The effect of these changes is that the greater part of the mesoderm is now arranged in two parallel longitudinal bands along the ventral surface of the embryo; these bands being connected in the middle line by a thin portion consisting of two layers (fig. 22). Fig. 21 is a transverse section through the ventral half of an ovum at this stage.

The two longitudinal bands now begin to be constricted off into the mesodermal somites. The latter are formed from before backwards and their position corresponds with that of the future segments of the body. The number of somites thus formed is eight, corresponding to the eight segments with

which the embryo is finally hatched. The somites are at first solid, but a cavity appears in them at a later period.

The ectoderm of the ventral plate now alters its character, the cells becoming more pointed and much more closely packed together.

From the Formation of the Stomodæum and Proctodæum to the Hatching of the Embryo.

Early on the ninth day the stomodæum is formed as an invagination of the ectoderm near one end of the ventral surface. Shortly after the first formation of the stomodæum the proctodæum appears as a shallow, somewhat wide invagination near the end of the ventral surface.

The body segments, already established by the segmentation of the mesoderm, now become more apparent, each being marked by a deep transverse furrow in the ectoderm (figs. 24, 25, 28). Fig. 12 is a section taken longitudinally through the embryo, and shews the stomodæum, the proctodæum, the eight mesodermal segments, and a single ectodermal furrow close behind the stomodæum. Fig. 24 shows this first furrow under a higher power. (Zeiss c.)

The endoderm cells are still scattered within the yolk, but they are gradually becoming collected in the median line just below the mesoderm. The stomodæum and proctodæum become more deeply invaginated, extending a considerable distance into the yolk and at the same time the endoderm cells begin to form the mesenteron, arranging themselves round a central lumen. Fig. 27 shows the formation of the proctodæum and the hypoblast cells beginning to form the mesenteron.

At the end of the ninth day, then, the embryo is of a long oval shape, with a deeply invaginated stomodæum at the anterior end and a proctodæum not quite so deep at the other; the mesoderm is divided into eight segments ; a deep furrow in the ectoderm marks off the first segment which will eventually become the head, and the mesenteron is almost formed.

The changes which take place on the tenth day result in the embryo assuming its definite shape. These changes consist of the completion of the ectodermal segmention, the formation of the nervous system, and the formation of the ventral flexure. Eight segments, including the head, are marked off from one another by ectodermal furrows, the last segment being a long one, from which the anal segment will eventually be divided off. Each of these eight mesodermal somites has now acquired a cavity. This is shown in fig. 28, which is a vertical longitudinal section through the second segment on the tenth day.

The two layers are distinguishable, the somatic being chiefly concerned in the formation of the muscles of the limbs.

The ventral flexure now begins to be formed between the seventh and eighth segments. Its first appearance, shown in figs. 29, 30, is seen quite clearly from the outside through the chorion. Metschnikoff has described it as occurring on the tenth day in Strongylosoma, which hatched on the seventeenth day, in a more advanced stage than Julus terrestris is at the time of hatching.

The ventral flexure is first formed by a deepening of the transverse furrow which forms the division between the seventh and eighth segments. It is therefore first formed nearer the anal end of the embryo. As the furrow deepens and the embryo increases in size, the last segment grows in length. The furrow does not deepen in a direction at right angles to the long axis of the embryo, but in a slanting direction, as shown in fig. 14. The effect of this is that the end segment is bent round against the head segment. The eighth segment just referred to is considerably longer than any of the others except the head, and the tissues show a considerable difference to the tissues in other parts of the body. Even on the eleventh and twelfth days, when the nervous system is far developed in all other parts of the body, in the eighth segment the tissues are imperfectly differentiated, the nerve-cord not showing any ganglia but lying on the ectoderm as a thin cord not quite separated from it. At a later period of development the anal segment is constricted off from this

segment, and from its anterior part the future segments formed in later life are developed. Just before the first appearance of the ventral flexure when the body segments are fully formed, the embryo developes a cuticular envelope over the whole surface of the body. This may be seen during the first formation of the ventral flexure surrounding the body but hanging loosely from it. This envelope is the so-called amnion of Newport.

Just before the first trace of the transverse furrow which marks the beginning of the ventral flexure has made its appearance, the nervous system begins to be formed. The first traces of this consist in a thickening of the ectoderm on each side of the middle line. This is soon followed by the formation of a shallow furrow between the thickened parts; this longitudinal furrow corresponds with that described by Metschnikoff in Strongylosoma. Fig. 31 shows the furrow and the ectodermal thickenings. Fig. 32 shows a later stage where the nerve-cords are almost separated from the ectoderm. The bilobed cerebral hemispheres are formed first and the nerve-cords are formed from before backwards, the posterior portion not being complete till a considerably later stage of development.

The nerve-cords are widely separated, but are connected by a thin median portion. In later embryonic life they are closely approached to one another and almost form one cord.

On the eleventh day the embryo has increased considerably in size. The ventral flexure is complete and the animal lies with the long end segment folded closely against the rest of the body, the end of the tail being against the stomodæum. The nervous system is now completely separated from the ectoderm, and the ectoderm has now assumed its adult appearance. It now separates a second membrane like that which I have already described as occurring on the tenth day.

These two membranes I regard as equivalent to two moults of the animal. The nerve-cords have considerably altered its appearance; it has sunk deeply into the interior of the body except in the end segment and now lie closely beneath the

mesenteron. They are divided into ganglia, one pair being present for each segment of the body; from each ganglion a nerve is given off to the corresponding body segment. The sub- and supra-œsophageal ganglia are almost formed.

The splanchnic layer of mesoderm covers the mesenteron, the stomodæum, and proctodæum. The median part of the somatic mesoderm lies above the nerve-cord, between it and the gut; from thence it passes downwards to the body wall. This arrangement is shown in fig. 34, which is a transverse section through an embryo of the twelfth day.

Within the yolk, which is still present in great quantity in the body-cavity, there are present a number of cells remaining over from the hypoderm after the formation of the mesodermic keel, and the mesenteron. These cells eventually give rise to the circulatory system, to the muscles of the segments, in part at any rate, and to other muscles; they are therefore mesoderm cells. The lumen of the mesenteron is now continuous with that of the stomodæum and of the proctodæum.

Fig. 14 shows a longitudinal vertical section through an embryo of this age.

On the twelfth day the Malpighian tubes grow out of the proctodæum. Their lumen is from the first continuous with that of the proctodæum. They end blindly and are enveloped by the splanchnic mesoderm.

Fig. 34 is a transverse section through an embryo on the twelfth day. The section is taken through a ganglion in the posterior part of the body. It shows the two ganglia united by a narrow median part and each giving off a nerve to the ventral part of the body, where the rudiments of a pair of limbs can already be traced. The Malpighian tubes are also shown. This section also shows the body cavity divided into four compartments by means of thin layers of mesoderm. Late on this day the animal is hatched with only the rudiments of its appendages, and I propose to reserve a full description of the stage till a future time.

Literature.

But little work has been done on the early development of Chilognatha. According to Newport, De Geer was the first to watch the development of Julidæ (6). He observed that Julus and Polyxenus were hatched with three pairs of limbs and a fewer number of body segments than is possessed by the adult animal.

Savi was the next observer. In 1817, in a paper quoted by Newport (11) and which I have not been able to obtain, he said that Julus was hatched without limbs. The next observer was Waga. In 1840, he, in a paper quoted by Newport (11), states that the young Julidæ are completely apodal at the time of hatching. Gervais (8), the next observer, in 1844, gives a great deal of fresh information about the later development of Chilognatha, but has little to say with regard to the earlier stages before hatching. He tells us, however, that Glomeris marginata has three pairs of limbs before hatching; that Polydesmus complanatus has also three pairs when hatched.

Fabre (7) in 1855, investigated the development of Polydesmus, and describes it as having three pairs of limbs and eight body segments, including the head segment, at the time of hatching. He also investigated Julus aterrimus, and describes it as hatching on the fifteenth day, being then apodous and without any organ or appendage, and the shape of the body being reniform; five days afterwards, he tells us, that he observed the first traces of body segmentation, and that seven days after hatching the animal consisted of eight body segments and possessed three pairs of limbs.

Metschnikoff found that the young of Julus Morreletti were hatched with three pairs of limbs (9), while Newport found that in Julus terrestris the just hatched young only possessed the rudiments of three pairs of limbs, and faint traces of the antennæ. My own investigations, which were carried

out on the same species as Newport's, confirm his account. In my opinion the conclusion to be drawn from these different accounts is that in different species of Chilognatha, and even in closely allied species of Julidæ, the hatching of the embryo takes place at very different stages of development.

In 1841, Newport published his paper on the organs of reproduction and the development of the Myriapoda (11). This is the first paper containing any real information of the early stages in the development. On the first three days he describes the appearance of the yolk-spherules as seen through the chorion, and describes the whole contents of the egg as becoming firmer. On the fourth day he saw "a little granular mass on one side of the shell" which he was inclined to regard as the future being. He made no further observations till the nineteenth day, when he describes the ventral flexure of the embryo within the shell. On the twentieth day he was able to make out six body segments. On the twenty-fifth day the embryo was hatched.

I am inclined to think that the little granular mass which he describes on the fourth day was the first beginning of the blastoderm.

Nothing more was written on the early development of the Myriapoda till 1874, when Metschnikoff published his paper (9), which contains the greater part of what we know of Chilognath development. His fullest observations were made on Strongylosoma. He describes the segmentation, the formation of the blastoderm, the formation of the ventral plate, the ventral flexure of the embryo, the segmentation of the mesoblast, and of the body, and gives a full description of the later stages. As I shall have to discuss his paper in detail I will not attempt to give a fuller account of it here.

In 1877, Stecker published a paper (13) in which he describes the development of Julus fasciatus and several other species of Chilognatha. His account does not agree either with mine or with that of Metschnikoff. As his account has been fully criticised by Balfour (2), I will not refer to it here at greater length.

The above is a short account of the early literature of Chilognath development in the first stages of development, and as with the exception of Metschnikoff's paper the only bearing they have on my own work is to show that Chilognatha, even in very closely allied species, are hatched at different stages of development, I shall not refer to them again, with the exception of Metschnikoff's paper, which I shall mention further in the next section of my paper when discussing the bearing of my own work.

Summary.

With regard to the segmentation I have described, it will be seen that it differs considerably from that seen by Metschnikoff (9), who describes it as total; the ovum being divided into two, four, &c., segments. I saw nothing of such a division, nor does Newport, who observed the eggs of the same species as I did, record any such appearances. Newport's observations were made on the eggs of a species found in Madeira; that is in a hot climate; and as regards segmentation were not carried on by means of sections. As the amount and distribution of the food-yolk has a great influence on the segmentation, I think it probable that in my species the segmentation differs slightly from that in the species investigated by Metschnikoff. The difference, however, consisting in the external segmentation of the ovum is not, I think, a very important one. The segmentation of Julus terrestris, as I have described it, shows a remarkable resemblance to that found in Amphipods by Ulianin (14). He describes an external segmentation by means of shallow furrows formed in the surface of the ovum, which is composed in great part of food-yolk; in each space thus marked out, a large amœba-like mass of protoplasm provided with a nucleus is present; the division of these protoplasmic masses coincides with the formation of the furrows. When the blastoderm is just about to be formed the furrows disappear. At the close of segmentation, then the ovum is exactly like the ovum of

Julus terrestris inasmuch as the segments are represented by protoplasmic masses each of which is provided with a nucleus.

The formation of the blastoderm, as I have described it, agrees in the main with that given by Metschnikoff for Strongylosoma. According to this author, on the fifth day isolated masses of cells make their appearance on the surface of the ovum and spread themselves round it to form the blastoderm. He was unable to trace the origin of these masses of cells. What he saw was precisely what I have described in the earlier part of this paper.

The formation of the blastoderm in Julus is, then, such as is generally found in tracheate development.

The cells which at the conclusion of the blastoderm formation in Julus remain within the yolk, represent the endoderm, and have apparently been overlooked by Metschnikoff.

The mode of formation of the mesoderm almost exactly resembles that described by Balfour (16) for Spiders. According, however, to this author the greater part of the cells of the keel or ridge are derived from the ectoderm, whereas in Julus the ectoderm furnishes the greater part of them. Balfour states that the keel in Spiders is probably the homologue of the mesoblastic groove of the insect blastoderm. Patten (12) describes a median longitudinal furrow in the ventral plate of Phriganids which gives rise to the mesoblast and to part of the endoderm.

In Peripatus (17) the mesoblast originates from the primitive streak, i. e. from the indifferent tissue behind the blastopore, which can be called neither ectoderm nor endoderm. I think that all these structures are homologous.

With regard to the cells which, as I have already mentioned, are employed, neither in the formation of the keel nor at a later period in the formation of the mesenteron, but remain in the body cavity as mesoderm cells directly descended from endoderm—Balfour states that in Agelena, after the establishment of the hypoblast the cells remaining in the yolk are not entirely hypoblastic, since they continue for the greater part of the

development to give rise to fresh cells, which join the mesoblast. This is exactly what happens in Julus.

Metschnikoff has described the formation of the bands of mesoblast and their division into somites, but his figures are difficult to understand, as he has not drawn either the cell outlines or the nuclei.

The formation of the ventral flexure has been described by Metschnikoff, and, as I have already mentioned, was first seen by Newport. The flexure is, as I have before said, formed between the sixth and seventh post-cephalic segments; that is, it marks off from the rest of the body the long eighth segment in which the tissues are very imperfectly differentiated, and from which the anal segment has yet to be cut off. It is from this imperfectly-differentiated segment that the future additional body segments are formed in the later stages of development.

The mesenteron of the adult animal is, as was pointed out to me by the late Professor Balfour, marked with a series of constrictions corresponding with the external segmentation of the body, but no trace of such constrictions has as yet appeared.

The wide separation of the nerve-cords in the embryo has, so far as I know, not been pointed out by any author.

I propose to reserve for a future paper a more full description of the development of the nervous system, the circulatory system, and the segmentation of the embryo, as well as the account of the appendages and other points connected with the further development of the embryo.

The above investigations were entirely carried on in the Cambridge Morphological Laboratory.

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EXPLANATION OF PLATES XXIII & XXIV,

Illustrating Mr. F. G. Heathcote's Paper on "The Early Development of Julus terrestris."

Complete List of Reference Letters.

bl. Blastoderm. *c. in mes.* Cavity in mesoderm. *ch.* Chorion. *ceph. seg.* Cephalic segment. *c. p.* Central mass of protoplasm. *dors. ec.* Dorsal ectoderm. *ec.* Ectoderm. *f.* Follicular envelope. *gl.* Ganglion. *en.* Endoderm. *lon. fur.* Longitudinal furrow. *m.* Mesoderm. *Malp. t.* Malpighian tube. *mes. b.* Mesodermal bands. *mem. ex.* Membranous envelope. *mesen.* Mesenteron. *mes.* Mesoderm. *mes. hy.* Mesoderm cells directly derived from endoderm. *m. k.* Mesodermic keel. *nu.* Nucleus. *nucl.* Nucleolus. *p. netw.* Protoplasmic network. *pr.* Proctodæum. *proc.* Process. *r.* Ring. *r. ap.* Rudimentary appendage. *rem. k.* Remainder of keel. *seg.* Segment. *s. m.* Segmentation mass. *som. m.* Somatic mesoderm. *sp. m.* Splanchnic meso-

derm. *stom.* Stomodæum. *sub. gl.* Subœsophageal ganglion. *supraœ. gl.* Supraœsophageal ganglion. *y. h.* Yolk hypoblast cell. *y. sp.* Yolk-spherules. *v. e.* Ventral ectoderm. *v. f.* Ventral flexure. *v. p.* Ventral plate.

FIG. 1.—Section through an ovum while still in the ovary. (Zeiss, C.) *nucl.* Nucleolus. *nu.* Nucleus. *r.* Deeply-stained ring of first food-yolk. *f.* Follicular envelope of ovum.

FIG. 2.—Section of ovarian ovum shortly before laying. (Beck $\frac{2}{3}$ in.) *nuc.* Nucleolus. *nu.* Nucleus. *y. s.* Yolk-spherules.

FIG. 3.—Section through ovum on first day, shortly after laying. *ch.* Chorion. *y. sp.* Yolk-spherules. *c. p.* Central mass of protoplasm. *nu.* Nucleus.

FIG. 4.—The central mass of protoplasm has divided into two. *s. m.* Segmentation mass. *Nu.* Nucleus.

FIG. 5.—Section through an embryo on the third day. *bl.* Blastoderm. *s. m.* Segmentation masses.

FIG. 6.—Section through an embryo on the third day, rather earlier than Fig. 5. A segmentation mass has just appeared at the surface.

FIG. 7.—Early on the fourth day. *ec.* Ectoderm. *en.* Endoderm.

FIG. 8.—Fifth day, showing first formation of mesodermal keel. *ec.* Ectoderm. *en.* Endoderm.

FIG. 9 *a.*—Sixth day, transverse section through keel. *ec.* Ectoderm. *m. k.* Mesodermal keel.

FIG. 9 *b.*—Section through anterior end of same embryo.

FIG. 10.—Sixth day, keel spreading out into mesoderm. *ec.* Ectoderm. *en.* Endoderm. *m'.* Somatic mesoderm. *m.* First beginning of splanchnic mesoderm. *rem. k.* Remainder of keel.

FIG. 11.—Seventh day. *en.* Endoderm. *ec.* Ectoderm *v. p.* Ventral plate. *s. m.* Somatic mesoderm. *sp.* Splanchnic mesoderm.

FIG. 12.—Vertical longitudinal section through embryo of ninth day. *st.* Stomodæum. *seg.* 1. First body segment. *mes.* Mesoderm. *v. ec.* Ventral ectoderm. *pr.* Proctodæum. *dors. ec.* Dorsal ectoderm.

FIG. 13.—Longitudinal vertical section on tenth day. *st.* Stomodæum. *pr.* Proctodæum. *mesent.* Mesenteron. *mem. ex.* Membranous envelope. *seg.* Segment.

FIG. 14.—Longitudinal vertical section on eleventh day, taken a little to one side of middle line so as to pass through all the ganglia on one side. *supraœ. gl.* Supraœsophageal ganglion. *st.* Stomodæum. *pr.* Proctodæum. *mem. en.* Membranous envelope. *mes.* Mesoderm. *mesen.* Mesenteron. *n. gl.* Ganglia of nerve-cord. *n.* Nerve.

The above fourteen figures were drawn under a Zeiss's microscope with a

$\frac{2}{3}$ in. object-glass by Beck, and a No. 2 eye-piece by Zeiss. They form a complete rather diagrammatic series up to the time of hatching.

FIG. 15.—Nucleus of ovarian ovum just before hatching. Drawn under $\frac{1}{15}$ oil imm. Reichert. *nucl.* Nucleolus. *nu.* Nucleus. *y. sp.* Yolk-spherules.

FIG. 16.—Section through dividing segmentation mass on second day. ($\frac{1}{15}$ Reichert's oil imm.) *y. sp.* Yolk-spherules. *nu.* Nucleus. *nu.*[2] Nucleus of second segmentation mass.

FIG. 17 *a.*—Part of a section through first day ovum, showing protoplasmic network. ($\frac{1}{15}$ Reichert's oil imm.) *y. sp.* Yolk-spherules. *p. netw.* Protoplasmic network.

FIG. 17 *b.*—Part of a section through second day ovum, showing network. *p. netw.* Protoplasmic network.

FIG. 18.—Part of a transverse section through third day embryo, showing segmentation mass dividing to form blastoderm. *bl.* Blastoderm cells. *y. h.* Yolk hypoblast. *nu.* Nucleus. ($\frac{1}{15}$ Reichert's oil imm.)

FIG. 19.—Part of transverse section on fourth day, to show formation of mesodermal keel. *ec.* Ectoderm. *en.* Endoderm. *nu.* Nucleus. ($\frac{1}{15}$ Reichert's oil imm.)

FIG. 20 *a.*—Part of transverse section through sixth day ovum, to show keel. *ec.* Ectoderm. *m. k.* Mesodermal keel. *y. sp.* Yolk-spherules. (Zeiss, D.)

FIG. 20 *b.*—Isolated cells of the keel. ($\frac{1}{15}$ Reichert's oil imm.)

FIG. 21.—Part of a transverse section through an ovum on the ninth day early, to show thickened bands of mesoderm. *ec.* Ectoderm. *mes. b.* Mesodermal bands. (Zeiss, C.)

FIG. 22.—Part of transverse section through ninth day embryo, to show median portion between mesodermal bands. *ec.* Ectoderm. *sp. m.* Splanchnic mesoderm. *som. m.* Somatic mesoderm. ($\frac{1}{15}$ Reichert's oil imm.)

FIG. 23.—Isolated cells from transverse section on ninth day, to show connection between mesoderm and ectoderm. ($\frac{1}{15}$ Reichert's oil imm.)

FIG. 24.—Transverse section through part of an embryo on the ninth day late, to show the mesodermal segments. *stom.* Stomodæum. *seg.*[1] Furrow marking off the head segment. *m. seg.* 1, 2, 3, &c. Mesodermal segments. (Zeiss, C.)

FIG. 25.—Longitudinal section through cephalic section. *st.* Stomodæum. *ec.* Ectoderm. *mes.* Mesoderm. 1 *seg.* First segment. (Zeiss, D.)

FIG. 26.—Endoderm cell from an embryo of same date when the mesenteron is being formed. ($\frac{1}{15}$ Reichert's oil imm.)

FIG. 27.—Longitudinal section through the proctodæum in same embryo as Fig. 25. The mesenteron is just being formed. *pr.* Proctodæum. *en.* Endoderm. *m. seg.* Mesodermal segment. (Zeiss, D.)

Fig. 28.—Longitudinal vertical section through first post-cephalic segment of a slightly later embryo than Fig. 27. *som. m.* Somatic mesoderm. *ec.* Ectoderm. *mem. ex.* Membranous envelope. *sp. m.* Splanchnic mesoderm. *cav. in mes.* Cavity in mesoderm. (Zeiss, D.)

Fig. 29.—Longitudinal vertical section through part of a tenth day embryo, to show ventral flexure. *sp. m.* Splanchnic mesoderm. *som. m.* Somatic mesoderm. *v. f.* Ventral flexure. (Zeiss, F.)

Fig. 30.—Longitudinal vertical section through embryo rather later than Fig. 29, to show ventral flexure. *ec.* Ectoderm. *mes.* Mesoderm. *v. f.* Ventral flexure.

Fig. 31.—Transverse section through late tenth day embryo, to show nervous system. *sp. m.* Splanchnic mesoderm. *som. m.* Somatic mesoderm. *ec.* Ectoderm. *ec. th.* Ectodermal thickening. *lon. fur.* Longitudinal furrow between nerve-cords. *en.* Endoderm forming gut. (Zeiss, D.)

Fig. 32.—Ventral part of a transverse section through an embryo of the eleventh day, to show the nerve-cord and the Malpighian tubes. This section is taken in the posterior region, about the sixth segment. *som. mes.* Somatic mesoderm. *gl.* Ganglia. *Malp. t.* Malpighian tubes. *pr.* Proctodæum. *sp. mes.* Splanchnic mesoderm. *y. s.* Yolk-spherules. *v. ec.* Ventral ectoderm. *b. c.* Part of body cavity between the nerve-cord and ventral ectoderm. (Zeiss, D.)

Fig. 33.—Vertical longitudinal section through part of a twelfth day embryo, to show the stomodæum. *sub. gl.* Subœsophageal ganglion. *supraœ.* Supraœsophageal ganglion. *st.* Stomodæum. In this section the supra- and sub-œsophageal ganglia are not cut exactly in the middle line, and so appear smaller than they really are. (Zeiss, D.)

Fig. 34.—Transverse section through a twelfth day embryo in posterior region of body. *gl.* Ganglia of nerve-cord. *r. app.* Rudiment of appendage. *mes.* Mesoderm forming a partition to the body cavity. *pr.* Proctodæum. *Malp. t.* Malpighian tubes. *m. en.* Mesoderm cells in the body cavity derived directly from the endoderm.

All the figures were drawn by myself under a Zeiss's camera lucida.

On the Structure of the so-called Glandular Ventricle (Drüsenmagen) of Syllis.

By

William A. Haswell, M.A., B.Sc.,
Lecturer on Zoology and Comparative Anatomy and Demonstrator of Histology, Sydney University.

With Plate XXV.

Introductory and Historical.

In all the Syllidæ the regions of the alimentary canal (fig. 1) have a very characteristic structure and arrangement. The mouth opens into a buccal chamber, through which the protrusible proboscis is capable of being everted. The anterior portion of the latter — proboscis proper, "région pharyngienne" of Quatrefages [1]—is thin-walled, presents a circlet of papillæ, and, usually, a tooth or teeth, and is capable of being everted through the mouth until the tooth (which is a weapon of offence and not an organ of mastication, being furnished with a poison-gland) is thrust out in front in a line with the long axis of the body. On this follows a region—the gizzard (*g.*)—distinguished by the thickness of its muscular wall, succeeded by the glandular region into which one or more pairs of cæca (*c.*) open, and this in turn is followed by the intestine (*i.*), which forms by far the longest portion of the canal.

It is with the structure of the third of these regions—that which I call here the gizzard—that the present paper is concerned, a careful examination of the structure of that organ in several species having shown that it has been totally misconstrued by previous investigators.

[1] 'Histoire naturelle des Annélés,' tome i.

As regards its general form and appearance, this gizzard (fig. 1, *g.*) is a longer or shorter, straight cylinder, connected directly with the walls of the body only by two long, narrow bands of muscle (*pr.*), which pass from its posterior end forwards to become connected with the walls of the buccal segment, and act as protractors of the whole proboscis. Along each side of the cylinder runs a narrow longitudinal line which is lighter in colour than the rest of the surface. When examined under the microscope the whole wall of the organ is seen to be crossed by a series of fine parallel transverse lines, the number of which varies considerably in different species. The band-like spaces between these lines, again, are subdivided by a number of still finer cross lines into a series of (usually) squarish areæ, in the centre of each of which is a rounded spot which differs in colour from the rest. In preserved specimens these spots project as minute papillæ on the external surface of the organ. In some parts of the surface this arrangement of the parts is departed from, the areæ composing the transverse rows being hexagonal and being divided through the central spot by the transverse line.

In most of the interpretations of those appearances which have been published by previous observers I find a singular unanimity on one point, viz. the presence of transverse rows of glands, corresponding to the transverse rows of dots above referred to. In his masterly essay on all that was then known (1851) on the anatomy and physiology of the Annelida, Williams makes the following observations on the organ under consideration.

"To the œsophagus in all Syllidæ succeeds an elongated highly glandulated gizzard-like portion peculiar to and characteristic of this genus (fig. 59, *d*). The parietes of this portion are perhaps not dense and muscular enough to claim for it the character of a true gizzard. The glandules are arranged in transverse or circular rows, and communicate with the interior by means of a minute excretory tube or orifice."[1]

[1] "On the British Annelida," 'Report of the British Association for the Advancement of Science,' 1851, p. 234.

Milne-Edwards[1] refers to this part of the alimentary canal simply as the "portion charnue du pharynx."

Schmarda[2] in his account of Gnathosyllis diplodonta describes the œsophagus as leading into a long cylindrical stomach whose inner lining membrane is thickened into cylindrical tooth-like processes of which he counted forty rows.

In his general account of the structure of the Syllidæ,[3] M. Quatrefages merely remarks on the thickness of the muscular wall of the gizzard and notices the existence of the transverse lines. In his detailed account of the structure of Grubea fusifera[4] he says of the gizzard, "Les parois en sont très épaisses, robustes, et comprennent en procédant comme tout-à-l'heure, de dedans en dehors: 1° la muqueuse; 2° une couche musculaire à fibres longitudinales semblable à la précédente; 3° une couche fort épaisse, a fibres circulaires et dont la nature m'a laissé quelque doute, bien que je la regard plutôt comme musculaire que comme fibreuse; 4° une seconde couche à fibres longitudinales assez mince; 5° une couche de matière parfaitement homogène et transparente, au milieu de laquelle sont disposés, d'une manière très-regulière, de petits amas de granulations ayant l'aspect d'autant de glandules; 6° une couche sans organisation apparente qui semble n'être que la continuation de la gangue où sont noyés les singuliers corps glandulaires dont je viens de parler."

I do not find anywhere in the works of Claparède any detailed account of the structure of the gizzard in the Syllidæ, but, again and again, in describing various species of the family, he refers to the transverse rows of glands[5] in this part of the alimentary canal, which he calls "proventricule."

[1] "Règne Animal de Cuvier, édition accompagnée de planches," Explanation of Pl. xv.

[2] 'Neue wirbellose Thiere,' I, ii, p. 69.

[3] Op. cit., t. ii, p. 4.

[4] L. c., p. 38.

[5] See, for instance, the account given of the gizzard of Syllis aurita, Sphaerosyllis tenuicirrata, and others in the 'Annélides Chétopodes du Golfe de Naples:' in his earlier "Beobachtungen über Anatomie u. Entwicke-

By Ehlers[1] the name of "Drüsenmagen," or glandular stomach, is given to this organ, and, regarding its structure, he expresses the opinion that what were formerly looked upon as papillæ are really gland-sacs, which are arranged in a radiating manner embedded in the thick wall of the canal, and are filled with a dark, finely-granular material.

Marenzeller[2] adopts Ehlers's name for the organ, and describes the transverse rows of glands in the various species which he found in the Adriatic.

By Langerhans[3] the same view of the structure of the organ is taken. In his papers on the Annelida of Madeira and of the Canaries, that author refers to what I here call the gizzard as the stomach, and describes the rows of glands in its walls. The only author, so far as I am aware, who has correctly described the general structure is Eisig,[4] who points out that the characteristic elements are muscular and not glandular.

Observations on the Minute Structure of the Organ.

What have been referred to in the descriptions above quoted as glands, are, in reality, radiating columns of striated muscle, and glands are entirely absent. The reconcilement of this statement with those of previous observers—that is to say the explanation of the accepted error—is to be found in the fact that the radiating columns of striped muscle which make up the greater part of the thickness of the organ retain, in a certain sense, an embryonic character containing in their

lungs geschichte wirbelloser Thiere," the same author refers to those bodies as "papillæ."

[1] 'Die Borstenwürmer,' pp. 205 and 206.

[2] "Zur Kenntniss der adriatischen Anneliden," 'Sitzb. der k. Akad. der Wissensch.,' Band lxix (1874) and Band lxxii (1875).

[3] "Zur Wurmfauna von Madeira," 'Zeitschr. f. wiss. Zool.,' Band xxxiv; and "Ueber einige Canarische Anneliden," 'Nova Acta der ksl. Leop.-Carol.-Deutschen Akad. der Naturforscher,' Band xlii.

[4] "Ueber das Vorkommen eines Schwimmblasenahnlichen Organ bei Anneliden," 'Mittheil. aus. der Zool. Stat. zu Neapel,' Band ii, p. 261.

interior a core of granular nucleated protoplasm, which, the enveloping differentiated striped muscle being overlooked, might very naturally be regarded as being of glandular nature.

Like all the other organs in the perivisceral cavity, the gizzard of the Syllidæ (figs. 2 and 3) is enclosed in a layer of the peritoneal membrane. Beneath this a stratum of fine longitudinal and circular fibres of the ordinary non-striated muscle arranged in three layers. Then follows the middle layer (*b*), which is by far the thickest and contains the supposed glands, and, internal to this again, is a thin internal fibrous (muscular) layer with circular and longitudinal fibres followed by the thin epithelium of non-ciliated columnar cells, and, finally, the delicate chitinous cuticle. The thick middle layer contains in its outer portion a series of thin annular bands of non-striped muscular fibres (figs. 2, *c*, and 3, *e*), which are flattened in the direction of the long axis of the organ. These annular bands are arranged at regular intervals along the whole wall of the gizzard, and it is to their presence that is due the appearance of regularly arranged transverse lines which has been already repeatedly referred to. In a transverse section the middle layer is found along two lateral lines (seen on a surface view as the two light longitudinal lines) to be represented only by a raphe formed by the annular bands of non-striped muscle anastomosing with the inner muscular layer. The intervals between these ring-like bands are occupied by the ends of rows of radiating columns. In the species which I shall temporarily designate as Syllis *a*,[1] these columns (fig. 3, *b*) are $\frac{1}{75}$th of an inch in length, broadest at the outer end and gradually narrowing towards the inner extremity. The average breadth is $\frac{1}{175}$th of an inch. Between the fibres extends finely fibrous matter with nuclei, but this cannot be said to form anything that can be called a distinct sarcolemma on the surface of the fibres. The outer portion of the fibre is squarish in transverse section, the inner end rounded. Along the axis of the fibre runs a core of finely granular protoplasm

[1] All the species referred to in this paper are Australian members of the restricted genus Syllis.

with nuclei. There run along the fibre in nearly its whole length two clefts by means of which it is divided, save at the extremities, into two closely-approximated halves, each of which has an irregularly crescentic cross-section. The substance of the fibre (fig. 6) (i. e. all except the granular core), is composed of striated muscle-substance. The dim bands are broad, four or five times the breadth of the bright bands. The latter exhibit in their centre a narrow band having the appearance of a double or triple line of dark granules; this dark line is particularly conspicuous in fresh specimens, and is more strongly marked than in the muscular fibres of any Crustacean or Insect I have examined; it is also well seen in specimens preserved in alcohol and stained with hæmatoxylin (fig. 10), but varies in conspicuousness both in fresh and preserved specimens in different parts of the same fibre in accordance evidently with the state of contraction or relaxation of the part. In alcohol specimens teased and mounted in Farrant's solution (fig. 9) some of the fibres are found to have the bright bands in the form of more or less prominent ridges without a trace of the dark line. In fresh specimens, again, fibres will be found in which the dim zones are thickened so as to confer an annulated appearance on the fibre, the bright zones forming annular constrictions in which the dark lines are very conspicuous (fig. 6). Hæmatoxylin stains the dim discs and the dark lines, leaving the substance of the bright zones unstained. The employment of polarised light brings out strikingly the ordinary contrast between the optical properties of the dim and of the bright bands.

Longitudinal fibrillation is very distinct in all conditions of the fibres, and after treatment for a few days with weak chromic acid the constituent fibrillæ become very well defined and readily separable (fig. 11); each exhibits a very well-marked, fine, longitudinal striation. In such a preparation the continuity of the fibrillæ from one end of the fibre to the other is readily traceable, the transverse striations (i. e. the bright bands) being represented only by a simultaneous bend in the course of all the fibrils, together with an ill-defined

transverse line of granules which does not in any way interrupt the continuity of the fibrils.

By the action of acetic acid interfibrillar substance is brought into view in the form of narrow longitudinal lines of fine granules which sometimes extend without interruption through the bright disc. The same reagent also reveals in the substance of the fibre star-shaped bodies with numerous excessively fine processes.

On transverse section the fibres (fig. 4) present well-marked Cohnheim's areæ, which appear finely granular, this granulation being evidently the expression of the fine longitudinal striation of the fibrillæ.

The granular matter in the core of the fibre is coloured red in the fresh state, like nearly all the protoplasmic elements of the body of the annelid. In the form now under consideration (Syllis α) the relations of this granular matter to the differentiated muscle-substance which encloses it were not clearly made out, but in another species (Syllis β) the granules of the core towards the inner end of the fibre are seen to become arranged in longitudinal rows, and these, when traced onwards, are found, by coalescence of the rounded granules, to become converted into homogenous muscle-fibrillæ.

Syllis α has the transverse striations much more numerous and more strongly marked than any of the other species I have examined. In Syllis β and γ (figs. 12 and 13) the striations are fairly distinct, but are few in number, half a dozen or so in the length of a fibre; the fibre has a single longitudinal cleft, and therefore presents a C-shape in transverse section. The protoplasmic core is reddish yellow.

It is worth noting that this observation is in partial accord with the view of the constitution and development of striated muscle recently put forward by Wagener. [See Hofmann u. Schwalbe's 'Jahresbericht,' Band ii, and Pflüger's 'Archiv,' Band xxx, S. 511—535.]

In Syllis δ, some of the fibres are not distinctly striated; in other fibres the transverse marking is distinct enough—the striæ being much more numerous and closer together than

in Syllis *α*, though without the same strongly-pronounced character.

In Syllis *γ*, when the organ is strongly stained with hæmatoxylin, teased, and treated with glacial acetic acid, the interfibrillar networks of Retzius[1] are very clearly visible (fig. 7), having the appearance of longitudinal rows of extremely fine granules between the fibrils, and of thicker transverse lines (Krause's membranes, transverse networks of Retzius), usually two, but sometimes only one, in each bright zone. The fact that the transverse networks appear stained with the hæmatoxylin while the longitudinal do not, would seem to point to some difference in the substance of which they are composed. In the dim zone there is to be seen a broad well-defined transverse band, which is much more darkly stained than the rest. In such a preparation the fibrils will frequently be seen, when broken across, to tend to split up longitudinally into a leash of fine fibrillules (fig. 8), which coincides with the appearance of longitudinal striæ in the fibrils of Syllis *α* as described above.

Hollow polynucleated fibres of striated muscle-substance, similar in essential character to those above described, are found in various Vertebrates as an embryonic condition of the solid fibres, and in certain Insects and Arachnids as a permanent form. Simple (mononucleated) hollow fibres are found not unfrequently in various classes of the Vermes, and in some instances their substance may be transversely striated; but the occurrence in that group of compound or polynucleated fibres with transverse striation of a marked type is now recorded, as far as I have been able to ascertain, for the first time.[2]

[1] "Zur Kenntniss der quergestreiften Muskelfaser," 'Biologische Untersuchungen,' 1881; abstract in Hofmann und Schwalbe's 'Jahresbericht,' 1882. B. Melland, who seems to have entirely overlooked Retzius's paper, arrives at similar concluisons regarding the interfibrillar substance by means of the same method (staining with chloride of gold). 'Quart. Journ. Micr. Sci.,' xcix, p. 371 (1885).

[2] By Eisig, who in the memoir cited above, describes radiating cylinders of muscular tissue as constituting the greater part of the wall of the organ, the special nature of this muscular tissue has been overlooked.

Summary of results.

1. The part of the alimentary canal of Syllis previously regarded as a glandular ventricle is in reality of the nature of a gizzard, and contains no glands in its walls.

2. The structures supposed hitherto to be glands are hollow columns of striated muscle, which in Syllis *a* is of a strongly marked type.

3. The muscular elements of the organ retain an embryonic character, containing a polynucleated core.

4. The fibrils of the muscle are seen in Syllis β to be formed by the linear coalescence of rows of the large rounded granules of which the main substance of the core is composed.

EXPLANATION OF PLATE XXIII,

Illustrating Mr. William A. Haswell's Paper "On the Structure of the so-called Glandular Ventricle (Drüsenmagen) of Syllis.

Fig. 1.—Anterior portion of the alimentary canal of Syllis α, magnified. *œ.* Œsophagus. *g.* Gizzard. *c.* Cæcum. *i.* Intestine. *pr.* Protractor muscles.

Fig. 2.—Transverse section of the gizzard of Syllis α (semi-diagrammatic). *a.* Peritoneal and external muscular layers. *b.* Middle layer (striated muscle). *c.* Annular band of non-striped muscle. *d.* Inner muscular and epithelial layer.

Fig. 3.—Portion of a vertical longitudinal section of the gizzard of Syllis α, × 350. *a.* External muscular layers. *b.* Striated muscular tissue. *c.* Finely fibrillated intermediate tissue with nuclei. *d.* Central protoplasm. *e.* Annular band of non-striped muscular fibres. The epithelium is diagrammatically represented.

Fig. 4.—Transverse section of two of the muscle-columns of Syllis α in the outer portion, showing Cohnheim's areas. × 350.

Fig. 5.—Transverse section of one of the muscle-columns of Syllis γ, showing central protoplasm and Cohnheim's areas. × 350.

Fig. 6.—Portion of muscular fibre from the gizzard of Syllis α in the fresh condition. × 900.

Fig. 7.—Portion of muscle-column of Syllis γ, stained with hæmatoxylin and treated with glacial acetic acid, showing double transverse networks of Retzius and longitudinal networks. × 900.

Fig. 8.—The same slightly teased, showing fibrillules into which the fibrils tend to break up.

Fig. 9.—Portion of the striated muscle-fibre of Syllis α, preserved in alcohol, showing the projection of the bright substance as annular ridges.

Fig. 10.—Portion of striated fibre of Syllis α, stained with hæmatoxylin.

Fig. 11.—Fibrils of muscle of Syllis α, after treatment with 1 per cent. chromic acid for several days. × 900.

Fig. 12.—Entire muscle-column of Syllis γ, showing the external shape, the longitudinal cleft, and the central core.

Fig. 13.—Longitudinal section of muscle-column of Syllis β.

Carnoy's Cell Researches.

By

Arthur Bolles Lee.

With Plate XXVI.

In the year 1883 there was circulated in the book-trade a prospectus of a forthcoming work on comparative cytology, by J. B. Carnoy. This prospectus contained some very remarkable drawings of cell-structures. It was followed in 1884 by the first instalment of the work it announced, 'La Biologie Cellulaire.' This work contains a not inconsiderable quantity of new views, or modifications of old views, which, if true, are certainly highly important. In 1885 Carnoy launched a new journal, 'La Cellule,' intended to be devoted to the discussion of the entire biology of the animal and vegetal cell. The first volume of this collection contains a splendid monograph on the spermatogenesis of the Arthropoda, by G. Gilson, and an equally elaborate and searching investigation of the processes of cell division in the same sub-kingdom, by Carnoy.[1] All this painstaking work has hitherto been flatly ignored, in some cases for reasons that are manifestly deficient in vis logica, as, for example, that the author quotes the Talmud. I suspect that much of the neglect of Carnoy's work is due to reasons of this order; and, believing that many of his results are very valuable, I venture to ask to be allowed to give some account of them here.

Carnoy conceives of the cell-body after a fashion that does

[1] 'La Cytodiérèse chez les Arthropodes.'

not materially differ from received views. Its substance—cytoplasm—consists (Pl. XXVII, fig. 7, also fig. 19, and others) of a reticulum which encloses in its meshes a plastic granular liquid, which Carnoy calls the enchylema. This enchylema is the same thing exactly as the paramitome or interfilar mass of Flemming, and the reticulum of Carnoy is the same thing as the mitome of Flemming; the word reticulum being used instead of the less definite word mitome, because Carnoy holds that the mitome has in fact a reticular arrangement. That is to say, he holds a view more similar to the views of Frommann, Klein, and Heitzmann, than to the more cautious conclusion of Flemming, who admits the existence of the filamentar element, but does not admit that the reticular arrangement of the fibrils is the typical arrangement. Let me note in passing, that Leydig seems to find networks much more frequent than the plexiform, or radiating, or other arrangement of fibrils.[1]

Externally the cell-body is limited by a denser layer of cytoplasm called a membrane. It consists of more or less differentiated cytoplasm, and is frequently not separable in any way from the cell-body, in which case it only receives by courtesy the name of a membrane. It is frequently pitted; but Carnoy holds that the pits discoverable in it are not open pores, but are closed by more or less solidified enchylema or paramitome substance. I suppose that no one is concerned to deny the truth of this doctrine.[2]

The structure of the nucleus may be briefly sketched as follows: It consists essentially of a chromatic element, the (caryo-)mitome of Flemming; of a surrounding plasma, which Carnoy calls the "caryoplasma," and an enclosing membrane.

As to the chromatic or nuclein element: in its typical form (fig. 1, *bn.*) it appears as a more or less tightly convoluted single cord or filament, "cordon nucléaire," Balbiani; "Kernfaden," Strasburger; "boyau nucléinien," Car-

[1] Leydig, 'Zelle u. Gewebe,' 1885, pp. 1—11, p. 34, and particularly the admirable plates.

[2] Leydig's view (l. c., p. 12, sqq.) is more complicated, but not necessarily antagonistic.

noy. This cord possesses a structure, which may be demonstrated in large specimens, and which must be attributed to smaller specimens by analogical reasoning. This structure is that denoted by the term "boyau;" it is in fact a gut, consisting of a sheath and of contents. The sheath is structureless, and is achromatic. The contents are the chromatin of Flemming, which Carnoy more frequently calls nuclein, being satisfied as to the rightness of this chemical denomination. The contents have frequently a figured arrangement.

The gut may have a uniform calibre throughout; and this is taken to be the typical case. This is, according to my experience, the form most frequently met with in young nuclei. But very frequently, and especially in old cells, it is constricted at more or less equal intervals so as to become moniliform (fig. 2). The constrictions may become so deep that all the chromatin is forced out of the constricted parts and accumulated in the intervening bellies. Nuclei in this state are not infrequently described by authors as having no "reticulum," but only a number of "nucleoli" or "granules," the connecting bridges formed by the achromatic sheath between the globular chromatic swellings being easily overlooked. In senescent nuclei this process is very often carried much further, and the gut does actually become broken up into numerous elongated or globular segments. This process is perhaps often a pathological one, but in certain sorts of cells it appears to be perfectly normal. In ova it is the general rule, a rule to which there are very few exceptions. The gut splits into segments, and the segments run into drops or globules, the so-called "germinal spots."[1]

Whilst the granular and globular forms of the chromatic element are derived from the filamentar form by processes of constriction and segmentation such as these, chromatic networks,

[1] Besides Carnoy's figures, 'Biologie,' pp. 222, et seq., and the statement of recent writers bearing on this point, I may be permitted to refer to my paper in the 'Recueil Zoologique Suisse,' I, No. 4, in which this process is described and figured for the ovum of Fritillaria. The actual specimens are much more demonstrative than my figures in this respect.

such as those of the nuclei of Amphibia, are derived from the unsegmented filament. The filament is here fine, very long, closely convoluted. It is also extremely delicate, and where its convolutions cross and touch they adhere and fuse, forming nodal thickenings ("nucleoli" of some authors). But the filament none the less remains essentially autonomous, as is proved by its disentangling itself from this seemingly inextricable maze, and appearing in the well-known skein form or "convolution" of the first phase of karyokinesis. These networks of the Amphibian nucleus are real anatomical structures; but the chromatic networks, believed to exist in other groups, are for the most part either artifacts—adhesions of the loops of the filament being brought about by the reagents employed or by pressure—or they are mere optical simulacra of networks, brought about by insufficient resolving power in the objectives employed, or by faulty microscopic manipulation.

Is the doctrine just stated a true one? I have satisfied myself by my own observations that it is, and that the belief that the normal typical form of the chromatic element is that of a network is an "Idolon speluncæ," bred of too-exclusive dwelling in the cave of Salamandra. I think the question is mainly one of pure micrography, a matter which everyone must settle for himself with his finger on the fine-adjustment screw. Want of space forbids me to say more on the subject at present, and I can only remind the reader that the doctrine of a continuous nuclear filament is held, with certain modifications, by Balbiani, by Strasburger,[1] and by Rabl.[2]

The chromatic filament is embedded in an achromatic ground-substance, "Kernsaft," "suc nucléaire" of French writers, "caryoplasma" of Carnoy. What is the nature of this substance? Say the authors in general, it is a structureless juice. Says Carnoy, with Pfitzner, it has structure. It consists of a reticulum ("reticulum plastinien" of Carnoy, "para-

[1] "Ueber den Theilungsvorgang, &c.," in 'Arch. f. mik. Anat.,' 1882, and "Die Controversen, &c.," ibid., 1884.

[2] 'Morphol. Jahrb.,' 10ter Bd., 2 Hft., 1884.

chromatin" of Pfitzner), which encloses in its meshes a granular liquid ("enchylema" of Carnoy, "achromatin" of Pfitzner). It has, in a word, the optical and chemical properties of protoplasm. Carnoy maintains that it is protoplasm; cytoplasm is protoplasm outside the nuclear membrane, caryoplasm is protoplasm shut up within it.

How is this view justified? First of all by observation. The reticular structure of the plasmatic element may be made out by direct observation in those nuclei in which, as in Pl. XXVI, figs. 1 and 2 here, figs. 96—100, 118 of the 'Biologie,' and many others, the chromatic filament is not distributed throughout the whole nuclear space, but forms a small central clew, leaving the peripheral zones free. This kind of nucleus is somewhat uncommon, yet not so exceptional but that it is well worth while to look for it. Carnoy recommends for this purpose the testicular cells of *Lithobius forficatus*. I have seen this structure in the epithelium of the intestine of Musca, but am not sure that it can always be found there. It is not an artifact, as I have been able to prove by observation of living objects (larvæ of Syrphida). In such objects as these, and in many ova, if not in most, the existence of an achromatic mitome[1] is as directly evident as that of the cytoplasmic mitome. In common nuclei it may be made out by careful study of very thin sections, and sometimes by dissection (see fig. 2). Digestion is useful, and so is treatment by solvents of nuclein, such as carbonate of potash. And, secondly, this view can be justified dialectically. In the karyokinesis of certain nuclei the spindle may be observed fully formed in the interior of the nucleus whose membrane is still perfectly entire. In these nuclei, therefore (and the number of these cases hitherto observed is not inconsiderable), we are obliged to admit the existence in the nucleus of an achromatic element capable of forming the spindle-fibrils. And that this element is protoplasm is proved by its genesis. In every case of normal

[1] Carnoy says "network." I have preferred to say "mitome," because I am personally not satisfied as to the reticular nature of the arrangement of the fibrils that I see.

karyokinetic cell division, the chromatic segments of the daughter-stars, after retreating to the poles of the figure, are seen to be immediately embedded in cytoplasm; around them, but often at some distance from them (see fig. 19, and the explanation of it), the new membrane becomes established, enclosing both the segments themselves and the cytoplasm in which they are engulphed. In other words, at each successive division the elements of the dyasters pitch their tent on new ground; they surround themselves with a fence enclosing a portion of new territory, and this new territory is cytoplasm.

The nuclear membrane is formed after the same manner as the cell-membrane, and has essentially the same structure. That is to say, it is pitted but not perforated, and there is no communication of any sort between the interior of the nucleus and the cell-body. I pass over the chapter on nucleoli, in which there are some judicious remarks, and one injudicious one—the doubt expressed relatively to Balbiani's discovery of the insertion of the nuclear cord into the nucleoli in the "salivary" cells of the larva of Chironomus. We come to the more important matter of the Cytodieresis of Arthropods.

Direct Cytodieresis.—This is the only mode of division that is found in the adult somatic cells of Arthropods. It is by no means infrequent, and may be observed in the most various organs. It may also be observed in reproductive cells.

The process of division is in general extremely simple. The nucleus elongates, becomes constricted by a narrow or by a broad equatorial furrow, into which the membrane is inflected, and which deepens until the separation of the two halves is complete. The division of the nucleus is very generally, though not always, followed by the division of the cell-body (the reader will not omit to notice how important this observation is in the face of the summing up of Flemming—'Zellstz.,' &c., p. 354—to the effect that the direct division of the leucocytes of Siredon, described by Ranvier, is the only case of that mode of division yet proved). And the process is

in general a very simple one: an equatorial furrow is formed, and deepens until the cell is divided.

But the process is sometimes by no means so simple. In the cells of the Malpighian tubes of the larva of Aphrophora ('Cytodiérèse,' p. 229, and fig. 7) it may be observed that the cytomitome, which in the static cell has an evident monocentric arrangement, its fibrils radiating regularly from the nucleus to the periphery, takes on a dicentric arrangement as soon as the nucleus has begun to be constricted. And in the fat-cells of Arthropods the matter is much more complicated and deeply interesting. Here the nucleus divides by simple constriction, without the accompaniment of the slightest karyokinetic phenomenon, but the cytoplasm divides by means of a cell-plate. The plate (figs. 3 and 4) has the same constitution as a vegetal cell-plate. It is formed by the regularisation and thickening of the fibrils of the cytomitome. It makes its first appearance in the centre of the cell, and grows outwards towards the membrane. It may continue to grow out so till it meets the membrane, thus cutting the cell simply into two halves (fig. 4). Or it may delaminate, split at the edge into two flaps, which are reflected towards the poles, and grow outwards till they meet the membrane on two separate lines (fig. 3), thus dividing the cell, not simply into two halves, but into two spheroids, between which there remains a ring, of triangular section, of cytoplasm, that is not taken up into the bodies of the daughter-cells, but remains outside till it deliquesces and is reabsorbed. The plate is transformed into a permanent membrane in the usual way by fusion of its component granules.

Carnoy has studied these cell-plates in Libellula, Acridium, Morimus, Bombus, Geotrupes, Eristalis, and Simulia.

Indirect Cell Division.—Nucleus.—The first prophase of karyokinesis, marked by the passage of the chromatic filament into the loose skein form[1] ("lockere Knäuelform, Spirem,"

[1] I use the term "skein" for the "convolution" of Klein, as being shorter. "Knäuel" is, I think, best translated by "clew," or "clue,"

"forme pelotonnée") is common to all Arthropods. The segmentation of the skein takes place very generally after the manner in which it is known to take place in the typical examples of animal and vegetal mitoschisis; that is to say, the skein breaks up irregularly into segments scattered without order throughout the whole extent of the nucleus. But very generally also it does not break up into irregularly-shaped and scattered segments, but into parallel bars. The skein arranges itself in long loops regularly set in zones parallel to the axis of the future spindle, arranged, that is, like the ribs of a melon (fig. 15). These loops then thicken in the equatorial region, and thin out towards the poles. They thin out at the poles till they break there (fig. 16), and we get a system of free bows set on parallel zones round the axis of the nucleus. The bows become shorter and thicker, and, either retaining their longitudinal position or becoming inflected in the middle towards the centre of the nucleus, constitute, without further change of place, a mother-star ("couronne équatoriale") of straight or bent segments, as the case may be; that is to say, mother-stars like figs. 6 and 18.

This mode of segmentation occurs, together with that of segmentation into scattered fragments, in all classes of Arthropods.

The Mother-Star—"Couronne Équatoriale."—The segments resulting from either the one or the other of these processes arrange themselves at the equator in a group corresponding to the "Sternform" of Flemming. They may form a regular circle situated on the periphery of the spindle, or they may form a plate occupying the whole section of the spindle. The positions they may take relatively to the filaments of the spindle are very various. Straight segments are generally attached to their filaments by their whole length; the segment lies on its filament (figs. 6, 18). But they may be attached to

which is its linguistic homologue. But it appears better to keep "clew," for the tight "Knäuel" which I hold with Carnoy is the typical form of the resting chromatic element; using "skein" for the very peculiar expanded form known as the "convolution."

their filament by one extremity only, and stand out perpendicularly to the direction of the filament. In this case the extremity by which they are attached is bifid, as in the pollen-cells of Fritillaria (see Strasburger, 'Die Controversen,' &c., Taf. xiv, figs. 68, 69; or Flemming, 'Zellstz.,' Taf. viii, fig. 7). Slightly-curved segments are apparently attached to their filament by the back or side; in the case of deeply-curved or U-shaped segments it can be clearly made out that the filament passes inside the bend of the U.

Fission of the Segments—Metaphase ("dislocation de la couronne").—The separation of the mother-star into two groups which travel to the poles is generally, as in hitherto studied animals and plants, preceded or accompanied by fission of the segments. In the majority of cases this fission is longitudinal. Straight segments may split at one end and open out gradually. Slightly-curved and U-shaped ligaments may split simultaneously throughout their whole length and along a strictly median line; or straight or slightly curved segments may split along a median line that becomes sinuous at the extremities of the segment, cutting through the ends, not in the middle, but at the angles (figs. 6 to 10), so as to produce two diagonally opposite hook-shaped moieties. This curious mode of separation, which appears to be by no means uncommon, appears to me very interesting as forming a transition between strictly longitudinal fission and transverse fission. In some cases the fission is transverse. In Astacus, Scolopendra, and Forficula, Carnoy has observed the fact in the most positive manner, and in other groups has frequently met with appearances which lead him to regard it as very probable.

The moieties produced by any of these modes of fission are in some cases destined to travel to opposite poles of the spindle, in other cases not so. A kindred fact to this last is that the fission may be retarded, and take place at the poles in the Dyaster stage, as in the fig. 86 of Flemming's 'Zellstz.,' p. 258. It is by no means impossible that longitudinal division may in some cases be entirely absent. It is, at all events, now

quite certain that the tendency to regard longitudinal fission of the segments at the equator as an essential element in mitotic cell division is no longer in harmony with observed facts.

The two groups of chromatic segments generally proceed to the poles of the achromatic figure, each segment on its particular spindle-fibril, in the typical manner. But one remarkable case (testicular cells of Œdipoda cœrulea, and of an undetermined locust) shows that it is not essential that the daughter-stars should proceed to the poles, and that the daughter-nuclei should be reconstituted there. In the cells in question the spindle does not elongate, but, on the contrary, flattens out (fig. 11). The segments, arranged in two lateral groups, do not travel along the spindle-fibrils, but, without changing place on their fibrils, are thrown to one side by the depression of the spindle (fig. 12; see explanation of these figures). The daughter-nuclei are reconstituted on points situated on the equatorial plane of the figure. It will be remembered that in the course of his work on the maturation of the ovum of Ascaris, van Beneden found that in the formation of the polar globules the plane of division passes through the axis of the dicentric figure, and is therefore perpendicular to the equator; whilst in normal karyokynesis the plane of division coincides with the equatorial plane, and is perpendicular to the axis of the figure. And van Beneden concludes that, on account of this striking difference, the formation of polar globules cannot be assimilated to karyokinetic cell division ('Rech. sur la Maturation, la Fécundation, etc.,' 1883, p. 338). The above recorded observation of Carnoy seems to render this conclusion nugatory, and to suggest that the formation of polar globules, after the manner described by van Beneden, should rather be taken as a particular case of karyokinetic cell division.

Reconstitution of the Chromatic Filament.—This is brought about by the union of the segments of the daughter-stars end to end. This union seems to be effected in various ways. U-shaped segments may simply take hands with their right and

left neighbours; or they may arrange themselves in the star with one limb directed outwards, and the other hanging inwards directed towards the axis of the figure; then the bends of the U's curve in towards the pole, and both limbs bend in towards the axis (fig. 13), and unite with the limbs of two vis-à-vis; the internal limb of any U joining with the external limb of one vis-à-vis and with the internal limb of another (fig. 14). It will be seen that in this case the daughter-nuclei present a disposition that reveals the organic axis of the nucleus; seen from the poles, the chromatic filament has a radiate arrangement, and seen from the side it appears as loops running parallel to the axis. In some cells (Arachnida, some Crustacea) the daughter-nuclei preserve this structure permanently, and it is then seen that at the next division they divide along the same axis as at first, and in a plane parallel to that of the first division. (It will be remembered that Rabl has recently ('Morphol. Jahrb.,' 1884, 2 Hft.) shown that two poles can be made out in cells of Salamandra during the first prophase.) In some cases the union of the segments is devoid of any apparent regularity; the daughter-stars appear to break up and the segments to unite without order.

The reconstitution of the filament generally follows closely on the arrival of the stars at the poles. But it may be delayed until after the formation of the nuclear membrane. It is possible that in some cases, when successive divisions succeed one another very rapidly, the reconstitution of the filament may never be completed.

As to the Spindle.—The spindle is formed out of the karyoplasm. This highly important proposition has been asserted before. It is now definitively proved by the discovery of numerous examples of nuclei containing a fully-formed spindle within a perfectly intact nuclear membrane (figs. 15, 17, and 18). Carnoy has now observed some thirty of such cases, in which the persistence and perfect integrity of the membrane were so evident as to leave no room for the least doubt.

The fibrils of the spindle are formed by the rearrangement

and regularisation of the fibrils of the reticulum of the karyoplasma. They form a continuous, uninterrupted system; that is to say, they are not interrupted at the equator (as held by van Beneden, who describes the spindle as formed by two cones approximated by their bases); and they are not interrupted at the poles, but course entirely round the spindle. This may be directly observed in those nuclei in which, as in the case of Œdipoda alluded to above, the daughter-stars are situated on the equator, leaving the poles free for observation (fig. 12).

The Asters.—Just as the spindle is formed by a modification of the karyoplasmic reticulum, so the asters are formed by a perfectly similar modification of the cytoplasmic reticulum. Their rays are true fibrils of plastin, and do not merely consist of aligned cytoplasmic granules. Their formation out of the cytoplasmic reticulum has been directly observed by Carnoy, and is figured by him in many places, e. g. figs. 83, 213*a*, 301 to 304, and 309 of the Cytodiérèse." The degree of development to which the asters may attain is extremely variable. In fully-developed asters it may be seen, especially by the help of digestion, that they form a continuous system; that is to say, the ray-fibrils are continuous at the equator (fig. 16). The asters then form as it were a cytoplasmic spindle, enveloping, but not continuous with, the nuclear spindle.

Polar corpuscles (*cp.*, fig. 18) are found in all groups of Arthropods, but are not constant, and except in Myriapoda and Crustacea are decidedly rare. They appear to have an almost liquid consistence, and to be merely transitory modifications of the cytoplasmic enchylema. They appear to play no part of any importance in the processes of cell division.

Separation of the Daughter-Nuclei.—On the dissolution of the nuclear membrane the cytoplasmic enchylema rushes into the nucleus and mingles with the karyoplasma. The spindle elongates, and the daughter-stars are carried out into a more or less remote region of the cell-body. Here the new membrane is formed around them, enclosing a portion of cytoplasm, which henceforth becomes karyoplasm, and so much of the

spindle as lies within the area of the new nucleus. The rest of the spindle is cut off by the forming membrane and discharged into the cytoplasm, of which it becomes a constituent part. It does not dissolve, but is utilised in the formation of the cell-plate (where a cell-plate is formed) and in the reconstitution of the cytoplasmic reticulum.

Plasmodieresis.—The plasmodieresis of cells whose nucleus has divided by karyokinesis takes place in various ways, any one of which, however, is a faithful copy of one or other of the ways in which it takes place in cells whose nucleus has divided akinetically. All the modes of akinetic plasmodieresis are represented, and that very frequently, in karyokinetic plasmodieresis.

The simplest mode is that of simple constriction. This is found in all groups of Arthropods, and in some of them is the only mode that is found. But on the whole, if I understand the author rightly, it is by no means the commonest mode.

The commoner case is, that a cell-plate is formed. The plate may be "complete" or "incomplete." When it is complete it has the structure of a vegetal cell-plate; that is to say, it may be considered as being made up of two portions, an internal one formed by the spindle, and an external one formed by the cytoplasm. The former is the spindle-plate, "plaque fusoriale," the latter is the cytoplasmic plate, or "plaque completive." I pass over the details of the formation of such a plate; they are essentially identical with those of the formation of a vegetal cell-plate.

Complete cell-plates are by no means infrequent in Arthropods. But incomplete plates are of still more common occurrence. By incomplete plates is here meant, as in vegetal cytology, spindle-plates which do not give rise to complete cell-plates by the addition of the cytoplasmic plate.

A plate, complete or incomplete, having been formed, the separation of the daughter-cells may take place in very various ways. There may be cleavage of the plate, and transformation of its layers into new membrane, as in vegetal cells; and this appears to be very generally the case when the plate is com-

plete. Where the plate is incomplete, the cytoplasm divides by constriction, and the nucleus may divide by cleavage of the spindle-plate. But it does not necessarily so divide. Both incomplete plates and complete plates may be formed and not utilised, the actual division taking place by constriction, and the plates disappearing either during the progress of the constriction or before it has begun to be formed, or after it is completed (fig. 20).

Conclusions.—The work I have thus shortly analysed establishes some very important conclusions. Let me state them; premising that if they do not appear convincing, that is because the limits of my space have obliged me to suppress an important part of the details of the observations on which they are founded.

The phenomena of karyokinesis are highly variable and highly inconstant. None of them are essential. There is not a "phase," from the formation of the chromatic skein or "convolution" to the reconstitution of the chromatic filament in the daughter-nuclei, that may not be omitted with impunity. Starting from the most complex phenomena of total karyokinetic cell division, with their complicated and regular figures of the prophases, their multiplied fissions of the chromatic element, their orderly metakinesis, their orderly and complicated methods of reconstitution of the daughter-nuclei, their highly-developed spindles, spindle-plates, and cytoplasmic plates, we can descend gradually through forms of karyokinesis of increasing simplicity till we arrive at forms so degraded as scarcely to be distinguishable from processes of direct cell division. And when on the other hand we remember that modes of direct cell division have been described which possess some of the characteristics of indirect cell division, such as an imperfect spindle, a suggestion of a skein form of the chromatic filament, or well-developed cell-plates, we are forced to conclude that direct and indirect cell division are not two essentially distinct processes, but rather modifications of one and the same general process.

Secondly, this process is essentially identical in the animal

kingdom and in the vegetal kingdom. The discovery that cell-plates are of common occurrence in so important a group of animals as the Arthropods, throws down the last barrier that was supposed to separate the cytodieresis of animals from the cytodieresis of plants.

EXPLANATION OF PLATE XXVI,

Illustrating Mr. Arthur Bolles Lee's Report on "Carnoy's Cell Researches."

Fig. 1.—Epithelium cell, from the intestine of a maggot. *mc.* Cell membrane. *pc.* Cell protoplasm or cytoplasm, showing a reticulum enclosing a granular "enchylema." *pn.* Nucleolar protoplasm or "karyoplasm," showing also a distinct reticulum and granular enchylema. *mn.* Nuclear membrane. *bn.* Nuclein cord or gut, contracted into a tight clew in the centre of the nucleus.

Fig. 2.—Nucleus from a trachea of a maggot. Teased preparation. The chromatic cord is here moniliform and transversely striated. The nucleus has been stretched by the needle and shows the karyoplasmic reticulum (achromatic) drawn out into a sort of spindle.

Fig. 3.—Fat-cell of Acridium lineola. It shows a cytoplasmic cell-plate delaminated into two reflected layers at *b*. The triangular spaces in front of *b* and *ϕ* are filled with protoplasm which will be absorbed.

Fig. 4.—Fat-cell of Morimus lugubris. *pc.* Cytoplasmic cell-plate, entirely traversing the cell. *n.* Nuclei. *e.* Vacuoles with enclosed urates.

Fig. 5.—Fat-cells of an undetermined larva of Libellula. *c.* Necks uniting three cells, of which the two lower are dividing. *m.* Membranes produced by the cell-plates of the previous division. In the central cell, and at *z* in the lower cell, a cell-plate fully formed. In the lower cell, numerous cell-plates in formation.

Fig. 6.—This, and the four following, are mother-cells from the testis of Bacillus linearis. They illustrate the diagonal fission of the elements of the mother-star, or "couronne équatoriale."

Fig. 6. The segments are straight and solid.

Fig. 7. The nuclein has deserted the axis of the segments, which are now vertically-compressed annuli, having their centres filled with hyaloplasma.

Fig. 8. A dark axial line appears in the hyaloplasma. The arrows show the sinuous diagonal line along which the segments will split.

Fig. 9. The segments have split, and the resulting moieties have wheeled half round in order to place themselves on either side of the equator.

Fig. 10. The moieties have completed their half turn, have thickened by contracting, and are about to start for the poles of the spindle.

Fig. 11.—This and the following figure are mother-cells from the testis of Ædipoda cœrulea.

In Fig. 11 the segments of the mother-star have arranged themselves in two lateral groups, and the spindle has begun to flatten. Two asters.

Fig. 12. The two lateral groups have further separated in the equatorial plane. The spindle, now fully formed, is stretched out in the equatorial plane, as testified by one aster which still remains to indicate the organic axis of the cell. Note that the spindle-fibrils are continuous at the poles, the place at the poles being indicated by the one remaining aster.

Fig. 13.—Mother-cell from the testis of Crangon cataphractus. The two daughter-stars, seen a little obliquely, are composed of U-shaped elements arranged regularly round the poles, with the bend of the U's looking in the direction of the axis, and one limb of the U's directed inwards the other outwards. These limbs are now beginning to curve inwards towards the axis, and will continue to do so till they take hands with their right and left vis-à-vis. *e.* "Nebenkern" or accessory corpuscle, playing no part in cell division. *pc.* Cell-plate, complete.

Fig. 14.—Schema, showing how the chromatic segments of the preceding figure unite with their vis-à-vis. *e.* External limb. *i.* Internal limb.

Fig. 15.—This and the following figure are mother-cells from the testis of Harpalus griseus.

Fig. 15. The skein-form or convolution of the first prophase is arranging itself in long loops parallel to the direction of the spindle. Spindle and asters visible, though not complete. Note that the nuclear membrane is still intact.

Fig. 16.—More advanced stage of this process. The chromatic loops have taken on a perfectly regular arrangement parallel to the spindle, and have thinned out and broken towards the poles. The segments thus obtained will now contract and thicken, and without further change of place form a mother-star like that of Fig. 6 or Fig. 18. Note that the fibrils of the asters are continuous, at least in many instances, at the equator.

Fig. 17.—Nucleus of a testicular cell of Stenobothrus. Mother-star formed in the usual way by grouping of the scattered segments, resulting from the transverse fission of the skein. Nuclear membrane perfectly intact, enclosing a spindle, not indeed fully formed, yet very evident. Drawn from a

nucleus dissected out from the cell-body, so that there can be no possible cause of error as to the existence of the membrane.

FIG. 18.—Cell from the testis of Astacus. Stage of karyokinesis more advanced than last figure. Mother-star with straight segments placed longitudinally. Spindle completely developed. The punctuated nuclear membrane, *x*, is still perfectly intact. Asters in formation. At either pole, three polar corpuscles, *cp*.

FIG. 19.—Group of mother-cells from testis of Lithobius forficatus. *a*. Prophase, skein form. Spindle visible, nuclear membrane entire, asters forming. *b* and *c*. Anaphases. In *b*, daughter-stars; the cytoplasma has invaded the spindle. There is a complete cell-plate, consisting of the spindle-plate, *pn*, and the cytoplasmic-plate, *pc*. In *c*, more advanced stage, one of the daughter-stars has nearly reconstituted its membrane, that is, its nucleolar membrane; the nuclear membrane, if it form, will be formed at the periphery of the halo. There is a thick spindle-plate, and a cytoplasmic-plate that has delaminated at the edge into two reflected layers, as in Fig. 3.

FIG. 20.—Cell with two reconstituted daughter-nuclei, from the testis of the larva of Aphrophora spumaria. A spindle-plate was formed during the karyokinesis, but not utilised. The spindle was pushed aside by the constriction, which served to halve the nucleus, and the remains of it, bearing a well-marked plate, are seen (*sp.*) lying in the cytoplasm.

The Pleomorphism of the Schizophyta.

By

E. Ray Lankester, M.A., LL.D., F.R.S.,
Jodrell Professor of Zoology
in University College, London.

Some students of natural history are content, when the explanations of phenomena which they have advanced and the arguments by which they have supported those explanations are appropriated by other observers, to remain silent, trusting to the justice of future generations for the vindication of their claims. So far as my own experience goes, an active observer who should trouble himself to obtain honest treatment from all his contemporaries in regard to the significance of his published writings, might abundantly employ the latter half of his life in struggling with new writers for that just recognition of his efforts in earlier years in advancing the knowledge of this or that subject, which is the one reward desired above all others by those who have not attained to the heights of philosophic contempt for the regard and sympathy of fellow-labourers. I do not intend to largely employ my leisure in this pursuit, but there is one subject on which I am anxious once for all to establish the significance of my observations and reasonings published twelve years ago in relation to similar views advanced and accepted at this moment.

That subject is what is now spoken of as the pleomorphism of the Schizophyta or Bacteria.

The view that the genera then recently established by Cohn, viz. Micrococcus, Bacterium, Bacillus, Vibrio, Spirillum, and Leptothrix, are form-phases, or variations of growth of a number of "Protean" species of Bacteria, each of which may

exhibit, according to undetermined conditions, all or some of these forms, was definitely and precisely formulated by me in my memoir on "A Peach-coloured Bacterium," published in the 'Quart. Journ. of Micro. Science' in 1873. I distinctly recognised the existence of true species of Bacteria or Schizophyta, but I pointed out that these must be characterised, not by the simple form-features used by Cohn, but by the ensemble of their morphological and physiological properties as exhibited in their complete life-histories. I illustrated my conception of the Protean or pleomorphic character of Bacterian species by a reference to the similar character of the species of Calcareous Sponges, and I had in my mind also the closely parallel facts established by Carpenter in relation to the endless variety of forms of the Protozoic Foraminifera.

My view was no merely speculative suggestion, but was based upon a careful study of a remarkable peach-coloured Bacterium, which exhibited a wide range of forms, connected by intermediate forms, growing together in the same vessel, and linked to one another most unmistakeably by the fact that they all were coloured by a special pigment which I studied with the spectroscope, and to which I gave the name "Bacteriopurpurin." I observed this organism on many different occasions from various localities; I figured and described its various form-phases; I obtained some modifications of form by cultivation, but chiefly depended upon the association of the different forms, the presence of completely transitional forms, and the common bond of the pigment, for the view as to their nature which I put forward. I gave the name Bacterium rubescens to this pleomorphic, or, as I termed it "Protean," species. I gave an account of further observations on this organism in the 'Quart. Journ. Micro. Sci.,' 1876, pp. 27–40.

Cohn opposed my view as to the genetic connection of the various forms associated by me under this name, and, contrary to the established laws of nomenclature, substituted a manuscript name in one of Rabenhorst's collections (viz. roseopersicina), for the duly-published name applied by me to this organism. He further describes some of its form-phases,

already figured by me, as Monas okeni, Monas vinosa, and Rhabdomonas warmingii.

On the other hand, two years later, Dr. Warming, of Copenhagen ('Vidensk. Meddelelser. naturhist. For. i. Kjöbenhavn,' 1875), after studying the same organism and figuring many of its form-phases, adopted my view as to their nature, and the extension of that view to the Schizophyta generally. He says: "Les bactéries sont douées en réalite d'une plasticité illimitée, et je crois qu'il faudra renoncer au système de M. Cohn." In 1883 Dr. Neelsen, in his 'Studien über die blaue Milch" (Cohn's 'Beiträge,' vol. iii, p. 241), cites my views and their confirmation by Warming, and rightly contrasts them with the later views of Nägeli and Billroth, and with that of Lister, who conceived that certain Bacteria were developed from a filamentous fungus (Dematium fuscisporum). As the result of his investigation of the Bacterium cyanogenum of blue milk, Neelsen says: "Viel eher würde für unsern Fall der Ausspruch Lankesters zutreffend erscheinen, von dem Proteus-ähnlichen Organismus, dessen einzelne Erscheinungs-formen eine Serie von Adaptationen vorstellen."

In 1884 Prof. de Bary, of Strasburg, in his 'Vergleichende Morphologie der Pilze,' p. 511, says, in regard to the question of species among the different forms of Bacteria: "There exist two views on this subject which are, at any rate in appearance, totally opposed to one another. The first is, as I think erroneously, ascribed to Cohn. . . . Cohn distinguishes merely what we have above spoken of as form-genera and form-species. The other view in its most extreme form amounts to this, that all distinction of species among the Bacteria is denied, and all forms are regarded as modifications of a single species or whatever else it may be called, and these modifications can be transformed by cultivation into one another reciprocally. This view was (if we leave out of consideration older intimations of a similar nature) set up in opposition to Cohn's classification by Lankester in 1873, and by Lister; and in 1874 carried to such a length by Billroth, that he united all the forms of Schizomycetes known to him under one

collective species, his Coccobacteria septica. It received later a support through the views which Nägeli (1877) expressed in the words, 'I have investigated during the past ten years many thousands of Bacterian forms, and I could not maintain (if I except Sarcina) that there was any need for a separation into even two specific forms.' Nägeli, however, adds that he by no means maintains that all forms belong to one single species: it were a bold thing in his opinion to express a definite conclusion in a matter in which morphological observation and physiological experiment leave the investigator so much in the lurch. He expresses himself again in the same sense in 1882. He nevertheless is, when carefully considered, in agreement with Cohn's fundamental conception, since Cohn erected his form-genera and his form-species (the latter based on physiological properties) primarily in order to gain a provisional survey, and irrespective of the question (as he distinctly states) as to whether as thus distinguished they correspond to natural species.

"Nägeli's words above cited contain a pregnant criticism of the whole controversy, so far as it had then gone. Both parties failed to bring forward (as is especially the case in Billroth's book) the only certain basis for their opinions, namely, the strict observation of the continuity or the non-continuity of the forms or species in question. In the absence of this, our judgment could only remain suspended, more especially since the forms in question are minute, very like to one another, often mixed together, and consequently easily to be mistaken for one another in the absence of quite strict observation. Lankester certainly came somewhat nearer towards establishing a special case of strictly-observed continuity, since the forms of his Bacterium rubescens (Beggiatoa roseo-persicina) gave evidence of their connection with one another more clearly by their characteristic colouration. Strictly-made morphological and developmental researches are now to hand. They have demonstrated that the forms known as Cocci, Rods, Threads, &c., are phases of growth (Wuchsformen)."

Thus writes Prof. de Bary in 1884. To some extent I have reason to thank him for the recognition which he gives to my position in this matter. But I cannot think that he has given a correct statement of my relation to the conclusion which he finally adopts when he associates me with Lister, who derived Bacteria from Fungi, with Billroth, who massed all Bacteria under one collective species, and with Nägeli, who declared that he did not see grounds for distinguishing as many as two.

The view which I put forward in 1873 is *precisely* that which Prof. de Bary now espouses, and I think I may very rightly object to its being confounded with the extreme and exploded theories of other naturalists. As to the "strict morphological and developmental researches" which now have made my doctrine of the pleomorphism of the Schizophytes acceptable to Prof. de Bary, I beg to point out that they do not differ in *character* from my own researches on *Bacterium rubescens*. Prof. de Bary very properly cites the later researches of Cienkowski, Neelsen, Hansen, and Zopf, as the chief amongst those which have tended to establish that view as to the forms and species of Schizophyta which I promulgated in 1873. They have done so, *not* by affording us any stricter evidence of actual observation of change of form taking place under the observer's eye, but by multiplying cases similar (in regard to the kind of observation made) to that published by me in 1873, viz. observations of the juxtaposition and structural continuity of different forms, and of the co-existence with extremely divergent forms of abundant intermediate forms.

In relation to the attitude taken up by one of the above-named observers, I have something further to say. Dr. Zopf has made valuable researches on various Bacteria and on the Mycetozoa, and has published the best systematic account of each of these groups which has appeared. In his quarto memoir (Leipzig, 1882) on the Schizophyta, as well as in the smaller handbook which he has since produced, Zopf gives a reference to my memoir on "A Peach-coloured Bacterium."

He has himself repeated my observations on that organism, but he has entirely abstained from pointing out in the text of his work how far his observations are simply repetitions of those published eleven years previously by me (which they are almost entirely), and he has in the most exact details adopted the view as to the pleomorphism of Bacteria which I then put forward, and on precisely the same grounds, without stating that he had been anticipated by me in this respect.

Not only this, but Zopf actually goes out of his way to ascribe to me a view differing from his own, and one which I have never suggested. Either Zopf is writing about my views without having troubled himself to ascertain what they are, or he is purposely misrepresenting them, when he says ('Morphologie der Spaltpflanzen,' 1882, p. v) : "Die Annahme Billroth's und Lankester's dass alle Spaltpilzformen nur Einer einzigen naturhistorischen Art oder Gattung zugehören, lässt sich nicht aufrecht erhalten."

I think Dr. Zopf will find it difficult to bring forward a citation from any writing of mine in which I have hinted, even in the remotest way, that "all the forms of Schizophyta belong to a single natural species." Billroth's declaration on this subject was published a year after my statement of the pleomorphic nature of the numerous natural species of Schizophyta, and never appeared to me to have any foundation in a general botanical experience, but to be the result of the restricted observations of a pathologist.

To remove all possibility of further misapprehension, I may be allowed to quote my own words ("A Peach-coloured Bacterium," 'Quart. Journ. Micro. Sci., 1873, p. 410) :

"The series of forms which I have found in the growth of Bacterium rubescens leads me to suppose that the natural species of these plants are within proper limits 'Protean.' . . . The natural species among the Calcispongiæ have been shown by Haekel not to correspond at all with the series of forms distinguished by his predecessors. . . . It seems exceedingly probable that the same manner of regarding the Bacteria will have to be adopted, Cohn's tribes and genera

taking the position of an artificial or formal system, whilst the natural species must be based upon some of those more profound characteristics which Cohn has himself indicated to us in his divisions—saprogenous, chromogenous, pathogenous. The indications of natural species do not lie under our hands in the case of the Bacteria, but have yet to be sought out."

I have now, I think, sufficiently pointed out the position of my publication on Bacterium rubescens in the history of the modern doctrine of the pleomorphism of the Bacteria. It will accordingly be readily understood that I cannot contentedly see this doctrine referred to, as it was recently by my friend Dr. Klein, as "Nägeli's theory of the pleomorphism of the Schizophyta," since Nägeli's view was announced four years after my publication, and is not identical with that at present accepted by De Bary, Zopf, and others, which is, in fact, precisely that put forward by me in 1873. Some of the recently published books dealing with the cultivation of pathogenic Bacteria contain also a general summary of what is known as to the natural history of the group, and an attempt to classify the non-pathogenic together with the pathogenic species. The importance of the doctrine of the pleomorphism of Bacteria in relation to pathological inquiries cannot be overestimated. It is therefore to be desired that in future editions the authors of the books referred to above will give a correct account both of the history and present position of this doctrine.

Notices of New Books.

1. Methods of Research in Microscopical Anatomy and Embryology. By Charles Otis Whitman, M.A., Ph.D. (S. E. Cassino & Co., Boston, U.S. 1885.)

This is a thoroughly trustworthy and ably written treatise. Dr. Whitman has had the widest experience in microscopical research; he is, as is well known, himself an accomplished observer and manipulator, who has mastered, for the purpose of his own investigations, the most recent methods. His work is more especially valuable as giving a full account of the methods of research which have been experimentally arrived at by the zoologists of the Naples Zoological Station, where Dr. Whitman has spent some months. The contents of Dr. Whitman's treatise are arranged in two parts, the first embracing methods of a more general nature, such as preservative fluids, dyes, macerating fluids, fixatives, mounting media, the microtome with its appurtenances, methods of embedding, &c.; the second including special applications of embryological, anatomical, and histological methods. In the appendix are described some methods of injection, museum methods, and formulæ for most of the important reagents, &c.

2. The Microtomist's Vade-mecum: a Handbook of the Methods of Microscopic Anatomy. By Arthur Bolles Lee. (London: J. & A. Churchill. 1885.)

Mr. Lee's book contains a valuable and very extensive collection of recipes. The author says of it: "The collection of formulæ here brought together is, I believe, practically exhaustive; no process having any claim to scientific status having been rejected, nor any, I trust, unwittingly omitted. The inclusion of all of them," he continues, "is justified by the consideration that some one or other of them may perhaps serve, in some way that cannot now be foreseen, to suggest some new method of value." In this we are entirely in accord with Mr. Lee. The description of methods is in some cases reduced to a rather small compass, and there is little attempt on the author's part to state critically the relative value of the different staining fluids, embedding methods, &c., which he records. But that is quite consistent with the object of the book, which is to give an exhaustive series of references. We do not doubt that Mr. Lee's

book will take its place as the standard and authoritative work of reference for all original investigators in microscropic anatomy. It is conceived and executed in a thoroughly scientific spirit.

3. **The Rotifera, or Wheel Animalcules.** By C. T. Hudson, LL.D., assisted by P. H. Gosse, F.R.S. (To be completed in six parts. Part I. London: Longmans, Green, & Co. 1886.)

The discoverer of Pedalion and the veteran illustrator of the Rotiferan mastax have joined forces to produce what promises to be one of the most beautiful works on natural history which an English publisher has ever ventured to issue. The Rotifera have of late years been studied almost exclusively by English microscopists, and by none with such remarkable and profoundly interesting results as those obtained by Dr. Hudson. We shall be able to speak more fully of the work when it is completed, but at present may limit ourselves to a word of admiration for the plates of Floscularia. Each part will contain five folio plates, which the publishers would do well to issue in unfolded folio form with a wide margin, in a volume accompanying the letterpress, instead of doubling them up as at present. Every naturalist's library throughout the world must contain a copy of this beautiful book.

Note on the Presence of a Neurenteric Canal in Rana.

By

Herbert E. Durham,
King's College, Cambridge.

With Plate XXVII.

In the supplement number of this Journal (1885) Mr. W. B. Spencer says that he has found that the only communication which exists between the neural and alimentary canals in embryos of Rana is by means of the blastopore, and he therefore doubts whether, strictly speaking, a neurenteric canal is present in Rana.

In the long vacation I cut some longitudinal and transverse sections of embryos of Rana, and on examining them I found that there was a well-marked communication between the alimentary tract and the neural canal without the intervention of the blastopore. Upon this I set to work to confirm this result by cutting a large number of embryos into series of sections, both longitudinal and transverse. As the number of my own specimens was rather limited, Mr. Sedgwick kindly allowed me to choose any suitable embryos from the laboratory materials.

In all the embryos which I have cut, of an age shortly after the closure of the neural folds, I find that the opening of the canal which communicates between the neural and alimentary canals is separated from the blastopore or anal opening by a projecting mass of cells, so that I think there can be no doubt

that it deserves the name of neurenteric canal in the strict sense of the words.

I quite agree with Mr. Spencer that the figure drawn by Götte (*vide* Balfour, 'Comp. Embryol.,' vol. ii, p. 107) of a Bombinator embryo will not apply to Rana embryos; first, because the canal does not persist to so late a stage as that figured; and secondly, because the arrangement of the parts is quite different to any that I have seen in Rana; and thirdly, because the anus is not represented as open; but this is not of importance as the section may not be in the right plane for the actual anal aperture.

I agree with Mr. Spencer that the blastopore persists as the anus in Rana. I may add, however, that I have one series of sections of an embryo Rana in which there is no blastoporal opening whatever, but I am inclined to regard this as pathological as I have so many other embryos which apparently are of the same age, and which possess a very well-marked aperture, with both the position and appearance of the blastopore.

Figs. 1 and 2 are the posterior ends of median longitudinal sections of an embryo of Rana; of these, fig. 1 passes through the blastopore, while fig. 2 includes the communication between the neural and alimentary canals.

Figs. 3, 4, and 5 are similar sections of another embryo; figs. 3 and 4 show the relations of the blastopore and the commencement of the neurenteric canal (*dn*); fig. 5 the latter canal and the posterior end of the neural canal. The neural canal (*nc.* in figs. 3 and 4) is continuous with that in fig. 5 (also marked *nc.*).

Fig. 6 is from a transverse section at the level of the blastopore; it shows the cavities of the neural and neurenteric canals and of the blastopore. A few sections further towards the posterior end the lumen of the neural canal is seen to be continuous with that of the neurenteric canal, and further forwards the latter is connected with the main gut cavity. The broad part of the neurenteric canal remains, and is very obvious for some time after the closure of the actual communication with the neural canal.

Continued Account of the Later Stages in the Development of Balanoglossus Kowalevskii, and of the Morphology of the Enteropneusta.

By

William Bateson, M.A.,
Fellow of St. John's College, Cambridge.

With Plates XXVIII, XXIX, XXX, XXXI, XXXII, and XXXIII.

Preface.

The following paper is descriptive of the figures which illustrate my concluding account of the morphology of the Enteropneusta.

As an abstract of some of these facts was given with the first paper on "Later Stages," &c., a certain amount of repetition has become unavoidable.

Owing to the relation of the parts described in that paper to other parts which were not then described, it has unfortunately become necessary to refer to figures in that paper on the present occasion; for this reason, the figures of the two papers have been numbered consecutively.

In the 'Quarterly Journal of Microscopical Science' for April, 1884, and April, 1885 (Supplement), I gave an account of the general development of B. Kowalevskii. The second of these papers contained a description of the later development of the notochord of this species, and a comparative account of that organ as found in other species. On the present occasion the remaining organs will be similarly dealt with.

Since the publication of these papers I have been able to make some further observations on the histology of the fresh tissues of the Brittany species (B. salmoneus and Robinii). For this opportunity I am indebted to the kindness of the directors of the Zoological Laboratory stationed at Concarneau, Finistère. And especially my thanks are due to Dr. Chabry for affording me these facilities.

The Skin and Nervous System.

The skin of all the species is entirely ciliated.

In the fresh condition I have chiefly studied it in B. Robinii, and it will be better first to describe its features in this form. Its structure is best seen by killing the tissue in a mixture of one part of 1 per cent. osmic acid and one of sea-water, then washing with sea-water, and staining with picrocarmine. This tissue on being teased out in glycerine shows the structure figured in figs. 76 and 77. The cells are very long, and most, if not all of them, extend the whole length of the skin (cf. fig. 75). The heads of these cells in the natural living state are closely in contact with each other, but on pressing out the tissue both in living and also in preserved specimens these heads may be stretched away from each other, but each remains attached to its neighbours' by more or less regular anastomoses. It thus is brought about that the surface of the skin is made up of a sort of honeycomb of tissue, each of the nodes being the outer end of an ectoderm cell. The cells are very difficult to separate finely, but the skin may easily be broken up into small rectangular pieces. On separation each cell is very thin; its outer end is slightly pyramidal, and is continued into a thin fibre which gives off anastomoses with adjacent cells and dilates at intervals. In one of these dilatations, generally the last, the nucleus is placed. Below this point the cell is continued into a very fine filament which may be traced for some distance. Many of these filaments terminate in small round knobs, which are possibly due to reagents.

In sections of hardened specimens these filaments may be

followed into the layer of nerve-fibre, which is always more or less developed at the base of the ectoderm cells over the whole body. These cells compose the larger part of the skin of the proboscis and collar. Amongst them are distributed cells which probably secrete mucus, &c. These cells are of several kinds. First, in the skin of the proboscis are large goblet cells whose nucleus alone stains (fig. 75, *mu'*). Next, in the skin of the back of the collar and of nearly all the rest of the body excepting those parts in which concentrations of nervous tissue are found, almost the whole tissue is made up of large cells full of some substance probably lubricating also, which does not stain. These cells are sufficiently represented in figs. 72 and 72A, which are, however, from B. minutus. In parts of the skin which are of this kind the long cells of the ectoderm are comparatively few in number, and thus the skin has a spongy consistency which is very characteristic. This is true of the skin behind the collar in B. minutus, B. salmoneus, and B. Robinii. There is a general similarity between the skins of all these forms, and probably their structure is the same as in B. Robinii. This statement, however, only rests on the evidence of sections, as no teased preparations were made of B. minutus. In the skin of the collar and proboscis especially a small number of nuclei may be seen in the higher layers of the skin. Whether these belong to young cells of the tailed series or of the secreting type was not determined. Another set of small, generally bifid secreting cells, are found in the proboscis skin; the contents of these cells are granular.

There is one other point of importance in treating of the skins of these forms, viz. the constant presence in teased preparations of large spindle-shaped cells (fig. 77, *c*). As the result of many observations it appeared nearly certain that these had really been broken off from the ends of the long ectoderm cells. Unless care was taken in the preparation this frequently happened, many of the ectoderm cells being broken and therefore without nuclei, and hence the probability that this was the origin of the spindle-shaped cells. Since these

fusiform cells are generally most abundant at that level of the skin at which the nuclei of the long cells are placed, the appearance is suggested that they form a second layer of ectoderm cells; but for the reasons above stated it seems likely that this is erroneous, and that there is no such definite second layer.

The resemblance between this skin and that of some Nemertines, e.g. Monopora vivipara (Salensky, 'Arch de Biologie,' 1884), is very close. In this animal the same spongy appearance is produced, and it is possible that the deeper layer of ectoderm may be capable of the same explanation.

The skin of B. Kowalevskii differs in some ways from that of B. minutus, &c., especially that of the trunk, in which the large goblet cells are comparatively rare. In all parts of the skin round, unicellular glands are more or less frequent, but their contents stain more or less deeply with hæmatoxylin, &c. These cells often fall out, leaving empty spaces. In the collar of B. Kowalevskii the skin is very thick and is full of very long cells (figs. 80 and 81) containing granular contents, which stain very deeply.

Fig. 79 shows a section of part of the proboscis skin in which the layer of nerve-fibre is very thick. In the upper part of this kind of skin there is a definite row of long nuclei which with some reagents assume a dice-box shape, probably due to preservation. To what extent these cells reach the whole depth of the skin cannot be affirmed, but many of them can be traced into fibres which run into the layer of nerve-fibre.

Nervous Concentrations in the Skin.—As has been already mentioned, in all the parts of the skin a greater or less quantity of unstained substance may be found in the base of the skin. The substance contains no nuclei (excepting a few in the nerve-sheath of the base of the proboscis), and may be seen, especially in fresh osmic acid preparations, to consist of fine fibres. Into it run the tails of ectoderm cells. In the next place fibres may frequently be seen running out of it through the basement membrane, and losing themselves

amongst the mesoblastic tissues. The question as to the nature of these fibres is one of great interest. They may either be mesoblastic fibres penetrating into the ectoderm as supporting structures, or they may be epiblastic fibres leaving the skin, in which latter case they are in all probability nervous.

Somewhat similar fibres have been described by Ludwig in the similar tissues of Asterias, and he is of opinion that they are connective tissue. The possibility, however, that these fibres in Balanoglossus are nervous is supported—firstly, by the fact that they always taper inwards and not outwards; secondly, that as a matter of fact, in B. Robinii at all events, the ectoderm cells may themselves be traced into tails of this kind; thirdly, the general absence of nuclei in the "punktsubstanz," for if these fibres are supporting cells, nuclei might be expected to be found in their course; fourthly, there is an à priori difficulty as to the nerve supply to the muscles in these animals, for, though the body of some of the species is very thick, no definite nerve-cords are to be found crossing the body cavities, with the exception of the "dorsal roots" mentioned hereafter. How, then, are the muscles innervated? It seems, then, at least possible that the nerve supply is derived directly from the skin, in which case the fibres leaving the "punktsubstanz" naturally suggest themselves as the transmitting agents. Finally, the view that these fibres are ectodermic is rendered likely from the fact that their origin may occasionally be traced from a very high level in the skin, though the appearance which is sometimes produced in sections as of their actual continuity with the undoubted ectoderm cells may not be quite reliable. In a few instances these fibres appear to anastomose with mesoblastic elements, though this cannot be quite definitely affirmed. On the whole, the balance of evidence seems in favour of the view that they are ectodermic. If this be correct the skin of Balanoglossus is to be regarded as a collection of sensory cells ending in long fibres, which may either be connected to the central nervous system, probably by the longi-

tudinal fibres of the "punktsubstanz," or may pass directly through this as motor fibres into the muscles.

The next point relates to the question as to the intervention of some third cell in their course functioning as a ganglion cell. In B. Robinii, in which the examination of this subject is most complete, as stated above, the occurrence of such cells could not be shown; but this is, of course, by no means conclusive in face of the antecedent probability of their occurrence. The "punktsubstanz," then, would mainly consist of afferent fibres passing to the central nervous system, and the motor fibres probably pass directly through it. As will be shown in the next paragraph its distribution agrees with this view.

In the account of the general development the central nervous system was shown to have arisen chiefly by a solid delamination from the skin, added to which its anterior, and to some degree its posterior, ends are being continually invaginated as growth continues, so that each end is tubular. This tubular form results not so much from the longitudinal closure of a tube as from a forward and backward growth of skin at the extremities of the delaminated cord. Soon after delamination histological differentiation occurred between the upper cellular and lower fibrous parts of the cord. While this was proceeding (2, *g. s.*) fibrous tissue was deposited to form the ventral cord at the point of this structure, which was most anterior (viz. the back of the collar). While this is proceeding the deposition of similar tissue in the region of the dorsal cord commences at the posterior attached end of the central nervous system. Next, the deposition of fibrous tissue extends itself forwards on to the proboscis, being first laid down in the dorsal middle line of the proboscis stalk (*v.* figs. 34 and 35, *pkt.*). On the appearance of the atrial fold the ventral and dorsal cords become united by a fibrous ring in the inner angle of the fold. This ring, therefore, may be supposed to bring up the fibres from the ventral cord to the central nervous system, which it enters at its posterior end, together with the dorsal cord (*v.* diagram, fig. 65).

The greatest concentration following upon these occurs in

the skin of the base of the proboscis. In the larva with four gill-slits (fig. 99, *P. rg.*) it is already well marked. Concentrations are formed in the line of the gill-slits (figs. 72A and 104), and slight fibrous anastomosing tracts run irregularly, following the line of the wrinkles from both the dorsal and ventral cords. These wrinkles taper towards both the cords and are permanent, being, in fact, limiting lines between patches of glandular cells.

Now, all these tracts of fibres are thickened as they approach the central nervous system, and dwindle peripherally. If this diminution were due to the continual separating of efferent fibres from the cords it would reasonably be anticipated that it would be greatest in the case of those parts of the body which lie behind the collar (i. e. behind the central nervous system); for these cords have almost the whole body to supply, but, on the contrary, it is the nervous sheath of the proboscis which presents the greatest concentration, and this continually thickens on approaching the collar, though the proboscis is conical and its base is towards the collar. This may be taken to show that this sheath of nerve-fibre is afferent, and is continually increasing in thickness owing to the incoming of sensory fibres from the ectoderm cells lying above it. Its sudden increase on the proboscis stalk is due to the sudden tapering of the base. This feature is particularly well seen in B. minutus. On any other hypothesis it would seem unlikely that this great deposition of nerve-fibre should occur in a region which is generally covered up by the anterior folds of the collar.

The Central Nervous System.—The changes occurring in this structure in B. Kowalevskii after its separation consist in an increase in size and in histological differentiation. As the result of these changes its anterior end comes to have the structure shown in fig. 60. Among the cells lining the anterior end of the lumen are always some few gland-cells. The cellular part of this cord is continuous, of course, with the cellular part of the skin, and the fibrous part or white matter, as we may call it, with the fibrous layer of the skin.

Behind the lumen it has the appearance shown in fig. 78. The white matter does not enclose the upper part of the cord. Above it are a number of pyriform cells, probably ganglionic, whose tails project into the white matter. Central to these the cells are more or less irregularly grouped into strands enclosing spaces. The histology of this central part of the cord is very difficult, and I have not been able to determine how these spaces are filled. In B. minutus (*v.* fig. 67) they are so definite as to make it certain that they are not due to reagents.

Among this loose tissue of the centre of the cord are remarkable stellate groups of cells (fig. 78, *stel.*) whose heads are thus placed radiating from a small lumen, which is generally sharply defined on three sides and usually irregularly bounded at some part of its margin. The nature of these stellate groups did not appear. They are commonest in the sides of the "grey" tracts, viz. at the points where the white matter is bent up (*v.* fig. 74, *b*). It is possible that the spaces thus enclosed may in some indirect manner communicate with the neural tube.

The histology of the cord is nearly the same in all the species. In B. Salmoneus and B. Brooksii, however, there is always a quantity of yellowish granules embedded in the central substance (on the analogy of Nemertines this substance may function like hæmoglobin). The shape of the cord in section varies in the different forms and in different parts of its course (*v.* fig. 74).

From the lower surface of the white matter of all species many fibres may be seen leaving the cord and losing themselves among the subjacent muscular tissues. In B. Kowalevskii alone no connection exists between the dorsal side of the cord and the skin. In B. minutus this is accomplished by three cords of skin substance. Their outsides are covered with a fibrous sheath (Spengel), and this is in connection with the fibrous layer of the skin. As Spengel has stated, these cords contain a more or less distinct lumen. I have not been able to trace this out upon the skin, though they occasionally

appear to lead to the cavities enclosed by the radiating cells. These cords I propose to term the dorsal roots. They occur in B. minutus, Robinii, salmoneus, and Brooksii. Their homology will be discussed when the other morphological questions arising out of these facts are treated of.

The histology of the rest of the nervous system has been sufficiently described.

The relations of the parts are explained by figs. 60, 64, 65, 67, 73, &c.

There are no special sense organs.

As the "dorsal roots" do not occur in B. Kowalevskii their development has not, unfortunately, been observed.

The Hypoblastic Structures.

The notochord has been described already, as also the mode by which the mouth comes to be anteriorly directed.

The cavity into which the mouth leads is lined by very thick walls (figs. 90, 67, &c.), composed of long cells supported by some intracellular substance, probably the same as that of the notochord. In B. Kowalevskii it leads continuously into the branchial chamber, but in the other species, in which the branchial chamber is separated by longitudinal ridges (fig. 91), from the lower cavity of the branchial region (which thus has the well-known figure-of-8 shaped cavity). The anterior end of the branchial cavity comes to be almost enclosed in the pharyngeal cavity. As the result of this on either side the branchial cavity projects as two blind horns, which are enclosed in the pharyngeal cavity.

The structure of the gill-slits has been sufficiently described by Kowalevsky, Agassiz, and Spengel.

To these accounts there is little to add. The figures 84 and 85 illustrate the mode by which their final structure is attained. It is practically impossible to follow their structure by means of transverse sections, but longitudinal sections and surface-views make them easily intelligible. Each gill-slit of B. Kowalevskii is U-shaped and surrounded by a skeletal secreted structure, as shown in fig. 85. In my last paper I

stated that, though the origin of these structures was uncertain, the balance of evidence favoured the view that they were hypoblastic. Since the above was written I have been led to regard them as more probably mesoblastic, owing to some of the appearances since observed. It should be noticed that the body cavity is continued into the valves always, but never into the bars separating adjacent gill-slits in which the bordering bars are in contact. This is due to obliteration of the cavity by the skeletal bars. This feature is very useful in distinguishing these parts in sections.

The atrial cavity must be described in this connection. As stated in the general account, its origin is due to the backward growth of the collar-fold to form an operculum. In B. Kowalevskii (*v.* fig. 88) it is more marked than in B. minutus, but in B. salmoneus the collar-fold does not reach as far as the first gill-slit, which consequently opens directly to the exterior (fig. 107). In B. Kowalevskii it covers about three gill-slits. (In fig. 88 only one gill-slit is thus shown; this is owing to the slight obliquity of the section.)

The relation of the opercular fold in B. minutus is shown in figs. 73 and 104.

The dorsal wall of the branchial chamber is thickened in the middle line to form a ridge (figs. 89 and 92). This ridge contains a groove in its posterior part. It is no doubt a supporting structure, and may conceivably be homologous with part of the backward extension of the notochord in other Chordata.

The digestive tract follows upon the branchial region. The branchial chamber ends in a short blind sac above it, and it is in this sac that the new gills are added after three pairs are formed (*v.* fig. 44). The walls of the digestive tract in B. Kowalevskii are thrown into an irregular spiral fold (*v.* figs. 82 and 108), which is not continued into the intestinal region as a definite feature.

The cells of the digestive region are arranged (fig. 82) in a single layer for the most part. They contain large granules

and bear a few long cilia. In the walls of the gut in this region are numerous blood-vessels. The lumen of the gut in this region varies greatly in size, probably with the digestive processes (cf. Salensky, loc. cit.), the liver being in B. Kowalevskii occasionally obliterated.

In B. Kowalevskii there is no distinct sacculation to form the liver, but in B. minutus the dorso-lateral walls of the digestive region are pushed out to form the characteristic liver outgrowths. These structures are not regularly paired. Their walls are full of secondary foldings (*v.* fig. 93). The cells lining these folds are similar to those of the digestive tract, containing large granules and fluid-looking vacuoles.

The skin covering these liver-saccules is very thin, and in B. salmoneus it may often be seen fused with the hypoblast, forming openings which place the cavity of the liver diverticula into actual connection with the exterior. The histological appearances are such as to leave no doubt that an actual fusion occurs. When the extreme softness of the tissue is remembered, it seems likely that these perforations may, in the first instance, be due to wounds which have healed so as to form fistulæ. [In a single case of B. minutus a fistula of this kind was found forming a perforation from the intestine to the body cavity. In this animal the fusion between hypoblast and mesoblast was quite complete.]

The liver of B. salmoneus is dark green in colour, and this colour is due to minute round granules or drops in the hypoblast. In B. Robinii the tint is generally dark brown.

The histology of the intestine, which is usually more or less diamond-shape, two of the angles being dorsal and ventral, is in no way remarkable. From the first the wall is formed of a single layer of cells, ciliated, and smaller than those of the digestive region (*v.* fig. 83). The anus opens immediately above the tail until this structure disappears, and then it opens widely in a terminal position (*v.* figs. 83 and 6).

The Tail and Anal Lappets.

The tail is present in the period between one and eight pairs of gill-slits. Its skin is full of unicellular glands. The third pair of body cavities are prolonged into it, and the mesentery between them remains. The anal lappets (fig. 3, *a*) also disappear with the tail.

Mesoblastic Structures.

Muscles.—The muscle-fibres of the proboscis are not gathered into bundles. They consist of circular, radial, and longitudinal fibres. The circular fibres are few in number, and chiefly occur in the external parts of the middle third of the proboscis.

The radial fibres are very few in B. Kowalevskii, but in B. salmoneus and B. Robinii they are common, and have a very characteristic appearance (*v.* fig. 94, *a*). Their peripheral ends are very long and fine, occasionally branching. Their central ends taper suddenly from a thick part containing a nucleus to a very fine fibre. These fibres are always plain fibres. Probably the peripheral ends are inserted into the skin, and the central end into the meshes of connective tissue which permeate the body cavity (*v.* fig. 79).

The longitudinal fibres of B. Kowalevskii are arranged in concentric rings, and united to each other by a peculiar connective tissue, which contains stellate cells with large nuclei. These concentric rings seem to be more numerous in old than in young animals, reaching the observed maximum of eight. This concentric arrangement is not a distinct feature until adult life is nearly reached. These fibres appear in section to have the same structure as those shown in fig. 94, *b*, which is taken from B. Robinii. The muscles of B. Kowalevskii were unfortunately not examined in the fresh state.

In B. minutus the longitudinal muscles do not form such definite concentric rings as in B. Kowalevskii, but all the mesoblastic tissues filling the proboscis cavity are broken up in preserved specimens into radial segments. This is not the case

in living B. Robinii, and hence is probably due to reagents in B. minutus; as, however, I have never had an opportunity of seeing the latter in the fresh state this cannot be affirmed.

In passing inwards from the outside to the centre of the proboscis the structures are thus arranged:

1. Ectoderm.—Ciliated tailed cells.
   Glandular cells.
   Nerve-fibres as a layer.
   Basement membrane.
2. Narrow tissue space crossed by ingoing fibres from ectoderm, and by supporting fibres in all directions, together with a very few circular fibres (*v.* fig. 51).
3. Tract densely filled with radial and longitudinal muscles (in B. Kowalevskii concentrically disposed in rings) and connective tissue.
4. The tissue space into which the central organs project.
5. The central organs:
   (*a*) Proboscis gland with its sac.
   (*b*) Heart.
   (*c*) Notochord.

The muscles of the collar body cavity in B. Kowalevskii are not gathered into bundles or definitely arranged, excepting those which are attached to the lateral rods of the axial skeleton (fig. 60). These large muscles are inserted into the back of the collar. The whole cavity between the pharynx and the skin, being originally second pair of mesoblastic pouches, becomes obliterated, being filled with muscles and connective tissue.

In B. salmoneus, B. Robinii, and B. Brooksii this also occurs, but in B. salmoneus (fig. 106) the longitudinal muscles are grouped into bundles. These bundles form two series, the one on the somatic and the other on the splanchnic side, and in the narrower parts of the cavity the groups of the two series dovetail into each other (fig. 106), being each gathered around a connective tissue septum projecting into the cavity.

These fibres in B. Robinii occasionally, after osmic acid, show a slight striping (fig. 94, *c*).

In B. minutus the longitudinal muscles of the collar lie in a layer immediately under the skin and under the pharyngeal wall. The cavity is crossed by many radial fibres, upon which some cells are placed, but is not so much filled up as in the other species.

The muscles of the third body cavity are not markedly different from those of the collar. In B. Kowalevskii alone a large muscular band runs along each side of the ventral nerve-cord, forming a projection from the body (*v.* fig. 108).

The perihæmal cavities are similarly almost filled with tissue, and always contain more or less longitudinal muscle-fibre. These are gathered into two bundles, and are inserted into the notochord sheath in the proboscis stalk. They are most developed in B. minutus, &c. (*v.* figs. 67 and 68).

The Mesenteries.—The dorsal mesentery persists throughout life in B. Kowalevskii and B. salmoneus. In the other species it disappears in the collar region. The ventral mesentery persists in the trunk in all species, but is always obliterated in the collar.

In B. minutus the body cavity of the trunk in the hepatic region is again divided in consequence of an attachment between the lateral angles of the diamond-shaped intestine to the body wall (*v.* fig. 93). In this position two large lateral vessels run.

As Spengel has stated, strands of connective tissue run in B. minutus from the body wall between the follicles of the ovaries, forming a sort of radial septa. These septa are probably not of morphological importance, beyond indicating the "accidental" way in which such septa may arise (cf. Polygordius, &c.).

All the body cavities are full of corpusculated fluid, as Spengel has observed. These corpuscles, when living, are full of bright granules and vacuoles, and exhibit amœboid movements.

The Proboscis Gland.—In B. Kowalevskii (fig. 47, *gls.*), at about the age of two gill-slits, a space appears in the proliferation of mesoblast lying dorsal to the anterior end of the notochord, when the latter is pushed forwards into the

anterior body cavity. This space is the first rudiment of the sac of the proboscis gland. Soon after its appearance it becomes enclosed in a membrane, which is added first at the posterior part of the sac (cp. figs. 45, 31, and 47). Its cavity is therefore a tissue space arising in the wall of the body cavity, and it is in communication with the body cavity by means of the interstices between the cells bounding its anterior end.

Its further development is involved with that of the heart, which had better be now described. The heart arises in animals with three pairs of gill-slits, as a horizontal split in the tissue between the notochord and the sac of the proboscis gland. Its walls are very thin (*v.* fig. 52). From the first it appears to contain blood, which is apparently non-corpusculated, and can be coagulated by reagents. Whether the heart is originally in connection with the dorsal vessel or not could not be determined. Its walls soon become slightly muscular (*v.* figs. 67 and 97), and the pulsations, which can be dimly discerned through the skin in the living state, are doubtless occurring in this vesicle.

After the formation of the heart a plexus of vessels in connection with it is formed among the mesoblastic cells covering the tip of the notochord (fig. 50). As this occurs the cells standing on the capillaries assume a pyriform shape, the sharp ends being fixed to the vessels and the wide ends free. These wide ends acquire a very transparent appearance, as though filled with fluid (fig. 49). These bunches of capillaries eventually acquire a great development and communicate with two larger blood-vessels (fig. 53, *b. v.*), and with a sinus in the periphery of the gland.

The sac of the proboscis gland anteriorly becomes filled up with a quantity of loose tissue, in which some granules of a yellowish colour are embedded.

In B. minutus these yellow granules are of much commoner occurrence (*v.* fig. 98). The capillaries of the gland are more regularly arranged.

In B. salmoneus the capillaries are still more regular, running parallel to each other to the periphery of the gland,

where they are united in a plexus of larger vessels (cp., figs. 95—97). The outer cells of the gland are modified to form a peculiar tissue (fig. 97). They are large cells, which stain deeply and have a nucleus usually on their outline. The cells standing on the capillaries contain some yellow granules, and larger granules or even masses of them are to be found in the spaces surrounding them.

The gland of the living B. salmoneus is light green in colour.

The nature of these glands is entirely obscure. These yellow granules occur amongst nearly all the mesoblastic tissues. In B. Robinii (collar) they may be found in the fresh state, presenting the appearance shown in fig. 100. They are never crystalline.

An attempt was made to investigate the chemical nature of these bodies, but with only negative results. They may, perhaps, be excretory, and it is possible that they are more or less removed by the proboscis pore and collar funnels respectively. This does not explain their presence in large masses in the trunk body cavity (*v.* fig. 93, *a*), from which no pore has been observed to open. Occasionally granules of this character occur in the ectodermic structures, suggesting that they are a product of the activity of all the tissues.

The proboscis pore was shown to arise at two gill-slits as a small vesicle in the skin of the proboscis stalk upon the left side (*v.* fig. 34); at three gill-slits it acquires an opening to the exterior, and at four gill-slits its tissue fuses with the lining of the left posterior horn of the anterior body cavity. (*v.* fig. 99), placing this cavity in communication with the exterior.

In B. Kowalevskii this pore is permanently on the left side of the body; in B. minutus, &c., it is median. [In my first paper on the "Later Stages," &c., p. 25, last line but one, B. minutus was written by mistake for B. Kowalevskii.]

The collar funnels arise as thickenings in the outer wall of the arterial cavity opposite the opening of the first gill-slit (*v.* fig. 101). These thickenings soon become perforated

(8, *g. s.*). At their origin they are simple conical funnels, but they soon acquire a crescentic lumen owing to a thickened inward folding of their outer wall. This is not conspicuous in B. Kowalevskii (cp. figs. 88 and 104). Their histology is sufficiently indicated in the figures.

As previously mentioned, the blood-vessels consist of (1) a dorsal vessel leading from the heart to the tail; (2) a ventral vessel running from the back of the collar to the tail; (3) in B. minutus a pair of large lateral vessels (*v.* fig. 93) in the digestive region. These are connected by plexuses in the skin and under the epithelium of the gut. In the operculum this capillary system of the skin forms a more or less definite circular vessel. In parts of their course these vessels are always more or less filled with a fibrous-looking substance, apparently cellular, which lines the walls (fig. 71). The generative organs lie in blood-sinuses derived from the subcutaneous plexus.

I stated (" Later Stages," &c.) that the branchial blood-supply resembled that of Amphioxus. From further observation I have come to the conclusion that this is a mistake, and that the vessels supplying the gills are all derived from the dorsal vessel, as Spengel has stated, being, in fact, merely the skin capillaries of the dorso-lateral regions. The main vascular trunks are all formed from the mesoblast of the first cavity and of the third pair of cavities. The capillaries under the skin and round the gut are formed in sitû in the mesoblastic walls in which they occur.

The Generative Organs.

The Ovaries.—The animals are all diœcious. The origin of the ovaries is not certain, but there is very strong evidence that they are epiblastic. At all events, from almost their earliest appearance, they are connected with the skin in the dorso-lateral regions (*v.* fig. 110). It is almost impossible to believe that an attachment of this kind is secondary, and I have never seen an ovarian follicle entirely separate in the body cavity.

Soon after its appearance it consists of a mass of loose round cells. A cavity next appears in its interior, as though due to a disintegration, and after the appearance of this cavity the cells bounding it develope into ova (figs. 111 and 112).

The egg-shell appears soon as a close-fitting membrane. The germinal spot is enclosed in a remarkably tough membrane in all the species examined. Though the ovaries are connected with the skin by ducts the ova are dehisced by the breaking away of whole follicles, which then disintegrate. In the branchial region of B. minutus there is a general correspondence between these ducts and the gill-slits, as Spengel has observed.

The testes are lobed masses placed in the same situation as the ovaries. The outer zone of each testicular follicle is made up of spherical cells (figs. 108 and 109, *a*), which contain several (? eight) deeply-stained dots. These cells are young spermatoblasts, and the dots, which increase in size in the spermatoblasts of the inner zone, are the heads of spermatozoa which are finally set free into the central cavity. Here they are arranged in curious strings, which wave above parallel to each other in preserved specimens (fig. 108). The testes, when mature, break up in B. Kowalevskii as masses, but in B. Robinii they exude from the skin as a yellow slime.

Mucus.—All the species secrete vast quantities of mucus when irritated. That of B. Robinii sets to form a mass of tough consistency, which collecting grains of sand forms a sort of tube. In this the animal can move slightly. The body of this species is very flat in the generative region, and is naturally folded up dorsalwards within the tube. The mucus of this form, which comes out after prolonged irritation, turns to a reddish-violet colour on exposure to the air, which is very characteristic.

In B. Brooksii, Robinii, and salmoneus the sides of the body are produced dorsalwards into flaps which nearly meet in the branchial region, and thus cover the gill-slits and dorsal nervous system.

EXPLANATION OF PLATES XXVIII, XXIX, XXX, XXXI, XXXII AND XXXIII.

FIGS. 64—112,

Illustrating Mr. Bateson's Paper on "The Morphology of the Enteropneusta."

Complete List of Reference Letters.

a. Anus. *al.* Alimentary canal. *at.* Atrial cavity. *bc.* ^{1}, ^{2}, ^{3}. The anterior, middle, and posterior body cavities respectively. *bg.* Rods bordering the gill-slits. *br. cls.* Border cells of proboscis gland (B. salmoneus). *b. v.* Blood-vessel. *C. N. S.* Central nervous system (*i.e.* the cord of the collar region). *Cap.* Capillaries of proboscis gland. *Circ.* Circular muscle-fibre. *Cl. f.* Collar funnel. *C. rg.* Ring of nervous tissue round the collar. *D. b. v.* Dorsal blood-vessel. *D. mes.* Dorsal mesentery. *D. n. s.* Dorsal nervous cord. *D. r.* Cords connecting central nervous system with the skin. *D. rdg.* Dorsal ridge of hypoblast in branchial region. *dig.* Digestive region of alimentary canal. *E.* Ectoderm. *fl.* Fold in wall of collar funnels. *g. s.* Gill-slit. *g. s.* ^{1}, ^{2}. First and second gill-slits respectively. *g. sc.* Lining of gill-sac. *g. sr.* Supporting rods of gills. *g. vs.* Germinal vesicle. *g. sp.* Germinal spot. *gl.* Proboscis gland. *gl. s.* Sac of proboscis gland. *gnl.* Granules in central nervous system of B. salmoneus. *gr.* Granules, probably excretory. *ht.* Heart. *int.* Intestine. *l. b. v.* Lateral blood-vessel. *l. rdg.* Lateral ridges separating the branchial chamber from the lower cavity of the gut in the branchial region. *l. msc.* Longitudinal muscle-fibres. *Lv.* Liver. *m. spz.* Spermatoblast cells. *Mo.* Mouth. *msc.* Muscle-fibres. *mu.* Mucous glands of skin. *mu'.* Goblet cells of skin. *mu''.* Long glands of collar skin of B. Kowalevskii. *n. cnl.* Neural canal. *Nch.* Notochord. *N. pr.* Neural pore. *n. sh.* Nervous sheath of proboscis. *O.* Opening of collar pores. *Op.* Operculum. *ov.?* Ingrowth of skin, probably an ovary. *ov.* Ovarian follicle. *ph.* Pharyngeal region of gut with thick walls. *per.* Perforation into liver saccule. *pkt.* Fibrous substance of the nervous system. *P. rg.* Ring of nervous tissue round proboscis. *ph. c.* Perihæmal body cavity. *Scl.* Liver saccule. *Sf.* Surface of skin with anastomoses of ectoderm cells. *Sk.* Skin. *Skr.* Sucker. *S. r.* Supporting rod of notochord. *Sp. vlv.* Spiral fold in wall of gut in the digestive region. *St.* Stripes occasionally seen in preserved muscle-fibres. *Stel.* Stellate masses of cells in central nervous system. *t. pr.* Tube of proboscis pore. *ts.* Testis. *V. bd.* Ventral band of longitudinal muscle of B. Kowalevskii. *V. b. v.* Ventral blood-vessel.

V. g. Nervous concentration in the line of the gill-slits. *V. msc.* Ventral muscles. *Vlv.* Valve of gill-slit. *V. n. s.* Ventral nervous cord.

Fig. 64.—Diagrammatic longitudinal vertical section of B. minutus, to show the arrangement of the nervous system. [The openings of the gill-slits are indicated, though of course not visible in a section of this kind.]

Fig. 65.—Diagram of nervous system of B. Kowalevskii as seen from the dorsal surface. The ventral cord and the ring round the pharynx are indicated in broken lines. The sheath of nervous tissue covering the proboscis is indicated by shading, as though the tissues were transparent. The gill-slits are shown on one side only.

Figs. 66—73 illustrate the structure of the skin and nervous tissues of B. minutus.

Fig. 66. Nearly median longitudinal vertical section of the middle third of the central nervous system, showing origin of two of the cords connecting central nervous system with the skin. Their union with the skin is not here shown. (*v.* Fig. 68.) Obj. A, long tube, oc. 2.

Fig. 67. Longitudinal vertical section through the side of the central nervous system, showing the relation of the neural and proboscis pores to each other, &c. The wall of the heart is cut in this section. As the section is taken through the side of the central nervous system its continuation into the dorsal nerve-cord is not visible. Obj. A, oc. 2.

Fig. 68. Transverse section of the central nervous system at end of neural tube. Obj. A, long tube, oc. 2.

Fig. 69. Transverse section of the central nervous system behind the neural tube, showing attachment of dorsal cord to the skin. Obj. A, oc. 2.

Fig. 70. Longitudinal section of the anterior end of the ventral nerve-cord. Obj. D, oc. 2.

Fig. 71. Transverse section of ventral nerve-cord. Obj. A, oc. 2.

Fig. 72. Longitudinal section of skin in lateral region. Obj. A, oc. 2.

Fig. 72 a. Transverse section of skin in the space between the gill-slits. Obj. D, oc. 2.

Fig. 73. Longitudinal horizontal section through the back of the collar, showing the relations of the peripharyngeal nerve-ring.

Fig. 74 (*a*, *b*, *c*).—Three sections taken through the anterior, middle, and posterior thirds respectively of the central nervous system of B. salmoneus.

Fig. 75.—Section of a wrinkle of the skin of the middle third of the proboscis of B. salmoneus. Obj. D, oc. 2.

Fig. 76.—Teased out osmic acid preparation of the skin of the collar of B. Robinii. The cells remain attached to each other by their heads. The

network, *sf.*, is formed superficially by the anastomosing heads, each of the nodes being the head of a cell. Obj. D, oc. 2.

Fig. 77 (*a* and *b*).—Cells of preparation similar to Fig. 76, more separated. (*c*) Spindle-shaped cells from lower layer of skin, probably broken off from cells resembling *a* and *b*. Obj. F, oc. 2.

Fig. 78.—Transverse section through middle third of the central nervous system of B. Kowalevskii. Obj. D, oc. 2.

Fig. 79.—Longitudinal section of skin of posterior third of proboscis of B. Kowalevskii. Obj. D, oc. 2.

Fig. 80.—Horizontal section through the skin of the collar of B. Kowalevskii. Obj. D, oc. 2.

Fig. 81.—Vertical section of the above. Obj. D, oc. 2.

Fig. 82.—Section taken tangentially to the flexure of the body of young B. Kowalevskii (8, *g. s.*), showing the spinal folding in the digestive region of the gut. Obj. B, oc. 2.

Fig. 83.—Longitudinal section of the tail of young B. Kowalevskii (4, *g. s.*). Obj. D, oc. 2.

Fig. 84.—Longitudinal section through the wall of the posterior region of the branchial sac, showing the relations of the valves and skeletons of the gills (B. Kowalevskii, 10, *g. s.*). Obj. A, oc. 2.

Fig. 85.—Diagrams of successive stages in the development of the gill-slits of B. Kowalevskii.

Fig. 86.—Macerated preparation of the gill-skeleton of B. Kowalevskii. Obj. A, oc. 2.

Fig. 87.—Longitudinal section of adjacent valve and gill-bar of B. Kowalevskii. Obj. D, oc. 2.

Fig. 88.—Longitudinal horizontal section through atrial cavity of B. Kowalevskii in the plane of the opening into it of the collar funnel and first gill-slit. Obj. B, oc. 2.

Fig. 89.—Transverse section through the back of the branchial sac of B. Kowalevskii (10, *g. s.*). Obj. A, oc. 2.

Fig. 90.—Vertical section of pharyngeal wall of B. minutus. Obj. D, oc. 2.

Fig. 91.—Vertical section of one of the lateral ridges, separating the branchial sac from the lower part of the branchial region. Obj. A, oc. 2.

Fig. 92.—(*a*) Transverse section of the dorsal ridge of the branchial region of B. Kowalevskii. (*b*) The same of B. minutus. Obj. A, oc. 2.

Fig. 93.—Transverse section through the junction of a liver saccule with the gut, through the back of the adjacent saccule (B. minutus). Obj. A, oc. 2.

Fig. 93 a.—Longitudinal section of some liver saccules of B. salmoneus One of these is perforated at its end. Obj. A, oc. 2.

Fig. 94.—Muscle-fibres of B. Robinii (osmic acid preparations). Obj. F, oc. 2. (*a*) Three isolated radial muscle-fibres from the proboscis cavity. (*b*) Two adjacent fibres belonging to the longitudinal system of the collar. (*c*) Two fibres from the same region as (*b*), which on treatment with osmic acid show an appearance of striping.

Fig. 95.—Section of the central part of the proboscis gland of B. salmoneus, anterior to the notochord. Obj. D, oc. 2.

Fig. 96.—Outer part of the proboscis gland of B. salmoneus, anterior to the notochord, to show the arrangement of the border cells (*br. cls.*). Obj. C C, oc. 2.

Fig. 97.—A radial segment of the proboscis gland of B. salmoneus in the region of the notochord. (For the relations of this tissue *vide* "Later Stages," &c., Figs. 51 and 52.) Obj. D, oc. 2.

Fig. 98.—Group of cells from the interior of the proboscis sac of B. minutus. Obj. D, oc. 2.

Fig. 99.—Longitudinal vertical section through the left side of the proboscis stalk of B. Kowalevskii (4, *g. s.*), to show the internal opening of the tube of the proboscis pore. Obj. D, oc. 2.

Fig. 100.—Concretions from the living mesoblastic tissues of the 2nd body cavity of B. Robinii. Obj. D, oc. 2.

Fig. 101.—Transverse section through the collar funnels and first gill-slit of B. Kowalevskii (10, *g. s.*). Obj. A, oc. 2.

Fig. 102.—Transverse section through the collar funnels and upper end of the atrial cavity of B. Kowalevskii (young adult). Obj. B, oc. 2.

Fig. 103.—Transverse section of B. minutus, passing through the internal opening of one of the collar funnels. (Hypoblastic structures indicated roughly.) Obj. A, oc. 2.

Fig. 104.—Longitudinal horizontal section of collar funnel of B. minutus at the level of the opening of the first gill-slit. Obj. D, oc. 2.

Fig. 105.—Transverse section through middle of collar funnel of B. salmoneus. Obj. B, oc. 2.

Fig. 106.—Transverse section behind Fig. 105. Obj. A, oc. 2.

Fig. 107.—Transverse section through external opening of the collar funnel of B. salmoneus. Obj. A, oc. 2.

Fig. 108.—Half diagrammatic transverse section of generative region of male B. Kowalevskii.

Fig. 109.—(*a*) Spermatoblast cells, forming the outer zone of the testicular follicle. (*b*) Spermatoblast cells, forming the inner zone of the testicular follicle. (*c*) Spermatozoa in the interior of the follicle. Obj. F, oc. 2.

Fig. 110.—Transverse section of young ovary of B. Kowalevskii (young adult). Obj. D, oc. 2.

Fig. 111.—Group of ovarian follicles of B. Kowalevskii, older than the above. Obj. D, oc. 2.

Fig. 112.—Ripe ovarian follicle of B. Kowalevskii. Obj. A, oc. 2.

PLATE XXXIII.

Diagrams.—Skin coloured light blue; nervous system, dark blue; hypoblast, light red; blood-vessels, dark red; mesoblast, green.

Fig. 1.—Blastosphere.

Fig. 2.—Gastrula.

Fig. 3.—Longitudinal vertical section through gastrula; blastopore being nearly closed.

Fig. 4.—Ditto; blastopore closed.

Fig. 5.—Ditto, later stage; mesoblast forming.

Fig. 6.—Longitudinal horizontal section through a somewhat later stage.

Fig. 7.—Longitudinal horizontal section through larva in Stage G.

Fig. 8.—Transverse section of collar of foregoing in plane of line *d d.*

Fig. 9.—Longitudinal vertical section of Stage H.

Fig. 10.—Transverse section of collar of foregoing in plane of line *d d.*

Fig. 11.—Longitudinal horizontal section of adult in plane of heart. (This plane would not really take in the perihæmal cavities, but their relations are thus made clear.)

Fig. 12.—Transverse section of foregoing in plane of line *d d.*

Fig. 13.—Longitudinal horizontal section of junction of collar and trunk in a larva of about 4 gill-slits.

Fig. 14.—Similar section to foregoing of adult, showing formation of operculum, atrial cavity, and collar funnels.

The Ancestry of the Chordata.

By

William Bateson, M.A.,
Fellow of St. John's College, Cambridge.

The Ancestry of the Chordata.

Preface.—In view of the facts relating to the structure of the Enteropneusta which form the subject of the accompanying paper and of those which have preceded it, it seemed necessary to attempt some analysis of their import and bearing upon morphological problems, and especially upon the vexed question of the ancestry of the Chordata.

But at the outset it was impossible to attempt such an analysis without first clearing the way by a discussion of the morphologic meaning of Segmentation. Since the Enteropneusta are essentially "unsegmented" animals and the Vertebrata are "segmented," this preliminary discussion was necessary. Moreover, having shown reason for not accepting the view that the vertebrate segmentation was of such a kind as to necessitate the existence of a series of segmented ancestors to account for it, it became also necessary to treat the whole question of the origin of segmentations of this class upon a wider basis. This must be the apology for the introduction into this paper of some matter and speculation not otherwise immediately relevant to the subject.

The decision that it would be profitable to analyse the bearing of the new fact in the light of modern methods of morphological criticism, does not in any way prejudge the question as to the possible or even probable error in these methods.

Of late the attempt to arrange genealogical trees involving

hypothetical groups has come to be the subject of some ridicule, perhaps deserved. But since this is what modern morphological criticism in great measure aims at doing, it cannot be altogether profitless to follow this method to its logical conclusions.

That the results of such criticism must be highly speculative, and often liable to grave error, is evident.

Part I.—The Segmentation of Amphioxus and the Vertebrata, compared with that of the Annelids.

From the time when the theory of descent in some form or other became generally accepted amongst zoologists, the question of the pedigree of the Vertebrates has been the subject of much speculation and controversy. The amount of attention which has been bestowed on this question has perhaps been greater than is warranted by the actual importance of the problem considered as a contribution to general biology; but when it is borne in mind that the question is that of the history of the human race, the fascination which has been found in it is not surprising.

Beyond, however, this more sentimental side, there is another source of special interest to be found within the terms of the problem itself; namely, that which is afforded by the obscurity of the solution; for when the relation of any one group to the rest of the animal kingdom is sought, in most cases there are some cardinal features of anatomy common to it and to some other group, which appear to point to some affinity between them. For example, the structure of the Tracheata at once suggests Crustacean affinities, while there is a strong apparent resemblance between the whole Arthropoda and the Annelids. Even a group so isolated as the Mollusca has points of obvious harmony with other groups as soon as the characters of the Trochosphere are known, and similarly with most other groups. Each and all of these "obvious" resemblances may be illusory, but still they furnish something

which, temporarily, is satisfying, and at least provides a point of departure for criticism. But in the case of the Chordata there are none of these common features. The three characters which unite them, the notochord, the gill-slits, and the relations of the nervous system, are limiting and exclusive, and without parallel in any forms outside the Chordate group. So strongly has this fact been felt by many of those morphologists who have already dealt with the pedigree of the group, that they have practically abandoned the attempt to find homologies for these features among the Invertebrates; for it is impossible to take seriously such suggestions as, for example, that the notochord may be compared to, generally, the sacs of the Capitellidæ, the "siphons" of any of various Invertebrates, the "giant-fibres" of Earthworms, or the crystalline style of Anodon. Each of these structures has been in turn suggested, together with many others, as offering something with which to compare the notochord. In the same way Semper argues that the vetebrate gill-slits have an obvious similarity to certain pores which he has found in the heads of certain Oligochæta (Nais), while other authors see a striking resemblance between them and the Chætopod segmental organ, and so on.

In seeking, then, for the proximate ancestors of Chordata, the Chordate features have been disregarded, and another character of the vertebrate animal has been selected as offering a more probable basis of operations. The character which has in this way been chosen as the point of departure is that of metameric segmentation. By thus setting aside the questions arising out of the notochord, &c., and speculating upon the segmentation of the body, the conclusion is soon reached that some Annelid was the immediate ancestor sought.

This view has found its chief exponents in Dohrn and Semper, and has been generally supported by Haeckel and by most of the popular exponents of evolution.

It would be unprofitable to recapitulate here the numerous morphological difficulties as to the primitive mouth, &c., which arise if this theory be received. Many objections of this kind

have been raised and have been variously replied to, and in this condition the matter rests. By those who support it, it is assumed that the common feature of segmentation is so binding and unique a property as to suffice to link together groups whose morphology is otherwise widely different.

In the following pages it is proposed to examine the propriety of employing the character of metameric segmentation as one of first importance in forming a phylogeny of this kind. And before referring to the evidence derived from the fact that the three characteristic features of Chordata are found in Tunicata and Enteropneusta, which are unsegmented forms, it will be best first to discuss the meaning of the phenomenon—"segmentation"—for if resolved into its elements it will be found to be by no means a peculiar feature of a few groups, but rather the full expression of a tendency which is almost universally present.

The term "metameric segmentation" has been used to describe several anatomical features, which reach their highest development in the Annelids, the Arthropods, and the Vertebrata. If an attempt be made to reduce this expression to its simplest terms it appears to mean, in the first place, that certain organs of the body are serially repeated from before backwards, and in the second place that, in the case of the Vertebrates and Annelids at all events, the body cavity is at some period of life divided into a series of compartments, each of which is closed off from its neighbours. But when a more precise account of this phenomenon is required, and when it becomes necessary to particularise as to which of the various organs of the body is thus repeated, difficulty at once arises from the fact that this repetition is irregular, and even within narrow limits may vary considerably. In the case of many of the errant Polychæts all the mesoblastic organs, together with certain apparently serially homologous parts of the nervous and digestive systems may recur for a seemingly indefinite number of times in one individual, or even the whole animal may be repeated in a chain, thus giving the highest expression to the phenomenon. On the other hand, as in Lumbricus,

&c., one or more of the mesoblastic organs may not be repeated; while in both Oligochæts and Polychæts there is a marked tendency to a division of labour between and specialisation of structure of individual segments or even regions of segments in various parts of the body. It thus appears that even among Annelids alone the fact of segmentation is not a circumscribed idea, but may include several phenomena which clearly differ from each other in degree, and possibly are also unlike in kind. For while in the case of Nais, &c., this repetition is complete, and is thus used as an obvious and simple mode of reproduction, yet in other worms it appears only to be concerned in increasing the length of one individual without adding to the number. Now, if these two conditions are merely various expressions of the same phenomenon the question at once arises as to which is its more primitive manifestation. Was segmentation originally a repetition of all the organs for purposes of reproduction, which process has become subsequently commuted into mere increase in bulk, or is this complete repetition to be regarded as the final term in a series of which the first was increase in bulk? Segmentation, as we know it, may clearly be viewed from either of these two standpoints. With regard to the Annelids, many authors have held that the former is the correct one; the question whether this is so or not cannot be discussed here, but in the case of the Chordata examination will show that their segmentation is of the latter class, and is the result of a summation of repetitions; and, being so, it is by no means a unique condition, which can unite forms otherwise unlike, as Chordata and Annelids, but is rather a result of the common tendency to repeat parts already present, which tendency occurs more or less in almost all animals. But before communicating the features of Chordate anatomy, which point to this as the mode of origin of the segmentation of the class, it will be best to establish the fact that repetitions of this sort are common, and to examine the comparative evidence as to the manner in which they occur. It will then be seen that segmentation on the plan found in the Vertebrates are really

extremely common, and appear to arise suddenly and in forms nearly allied to those in which they are not found.

Firstly, among the ciliated Platyhelminths a striking case is offered by Gunda segmentata, in which, as described by Lang, the diverticula of the gut, the testes, the yolk-glands, the tubules of the excretory organs, the transverse commissures, and the nerve-cord, are all regularly and synchronously repeated. Now, this case stands alone merely in the completeness of the repetition. All through the Turbellaria are to be found many instances of animals with great numbers of gut diverticula, with testes and yolk-glands scattered all over the body, with branched excretory systems, with anastomosing nervous networks, &c. Not only this, but instances are common in which some of these structures are repeated regularly, and others irregularly or not at all, as, for example, Polycelis pallida (Quatrefages), in which the ovaries are scattered and the testes are not, while the reversed condition is more frequent. It becomes probable that the repetitions of these organs did not phylogenetically occur simultaneously, but that repetition occurred at various times in each set of organs.

Again, among Nemertines in some species the saccules of the gut, the generative organs, and the circular blood-vessels are all repeated together and with great regularity, so as to produce a segmented whole. In other species these repetitions are not all formed or are more or less irregular, thus pointing to the fact that these repetitions have been acquired within the limits of the group. The development (*v.* especially Salensky, 'Arch. de Biologie,' 1884) precludes at once the possibility of the ancestral form of Nemertines having been "segmented;" hence they, together with the Planarians, offer a type of a high degree of repetition being acquired within the limits of a group. Nor do these forms alone exhibit this feature as one peculiar to themselves, for there are few groups in which it is not found. Even among Mollusca, which are, perhaps, the most typically unsegmented of all forms, the Chitons may be instanced as examples showing that such com-

plicated organs as shells may be repeated within the limits of a small group. Moreover, in some Chitons bunches of calcareous setæ recur along the sides symmetrically to the scutes, producing an appearance not far removed from that of Arthropoda.

Another case is to be found among the Nudibranchs, in which the liver diverticula, which are peculiar to and characteristic of the group, not only recur in an obviously segmental manner, but may be arranged in several ways among the Æolidæ, being in some (as Æolis papillosus, Æolis pulcher, &c.) arranged in more or less regularly paired oblique rows, while in others (as Dendronotus) the liver cæca stand in paired, arborescent tufts, which are as definitely symmetrical in their repetition as any system of organs of a Vertebrate. In cases of this kind the regularity of these repetitions is obviously secondary, and all the other anatomical features show no trace of segmentation, which constitutes the great interest of cases of this kind from the point of view of the present argument.

The cases which have been so far mentioned have all been selected from bilateral animals, with a definite long axis in the direction of which they move. But the belief that repetitions of this sort are of constant occurrence as a factor in effecting modifications of general form, derives most remarkable support from the facts of the anatomy of radiate animals, especially of the Echinodermata. From embryonic evidence it may be regarded as almost certain that these animals are descended from a bilateral ancestor, and that their present form has been since acquired. Whenever this change took place it came to pass in some entirely unknown manner that the various organs came to be repeated round a central axis. However this may have been brought about, the fact remains that the number of such repetitions did not become a fixed and definite feature common to all the divisions of the group. For while the number five appears to be the limit of the repetition in the Echinoidea, Ophiuridea, and Crinoidea, among the Asteroidea the arms of different genera have not the same number, nor do they necessarily occur in multiples

of any number. For example, while in the divisions Asteridæ and Asterinidæ the prevailing number is again five, among the Solasteride we find that the arms of Solaster may be thirteen or nine (as in S. endeca), in Heliaster from twenty-nine to forty. Not only is this true of living forms, but in the case of the fossil Cystidea the plates were irregularly arranged and the perforations of the feet scattered, and in the Blastoidea the basal plates were three, though bearing five radials and interradials. All these facts point to a history of the occurrence of repetitions among the various parts around a central axis. And perhaps more remarkable still is the extreme variability to be seen among individual members of living species.

For example, though Asterias rubens ordinarily possesses five arms specimens possessing six or seven arms are very common, while individuals with only four are not rare (the latter may possibly, however, arise from mutilation). In like manner specimens of Brisinga coronata are said to have from nine to twelve arms. Thus, in these cases the arms, with all the organs which they contain, may be spasmodically repeated as a mere individual variation.

All these animals move on the oral surface, and though, of course, the body may be regarded as arranged bilaterally round a longitudinal axis, yet in the locomotion of the animal this fact is not conspicuous (?) But in the Holothurians in which a long axis does again assume importance, though repetitions of this magnitude do not occur, yet there is a tendency for certain organs to arrange themselves in a series of longitudinal repetitions closely imitating segmentation. In this connection the Elasipoda (Holma Théel, 'Challenger Monographs'), which crawl about on the "trivial" surface in the direction of the long axis are of great interest. The body of these animals is long and flat, and its margins are produced into long processes, resembling parapodia, which are regularly arranged in pairs down the sides. The regularity of this arrangement is so great that some of the species figured by Théel might easily be thought at first glance to be segmented

worms[1]. Thus, in animals whose long axis has been suppressed, it appears that repetition may arise of most of the organs of the body radially arranged; next, that not only the specific but also the individual number of these variations is liable to great variations, pointing to the fact that the power to repeat in this way is one which may be easily called into action producing great differences of form.

It may also be observed in this connection that similar casual repetitions are frequent in the case of the Gonozooids of *Hydromedusæ*, in which animals also they are radially arranged. As in the case of the Echinodermata this is shown by the great diversity in the specific and individual number of those organs which are radially repeated. The latter may be seen, for example, in *Clavatella prolifera*. The Medusa of this animal creeps about on its tentacles, which are long and stiff, and which carry short suctorial processes on their oral faces which support the animal, giving it the appearance of an Ophiurid. The number of these tentacles and of the radial canals varies with age, from six to eight (Hincks). In the specimens which have come under my own observation in the undetached buds the number of these arms was five, while those of the free Medusa was generally six. The number of the organs in *Cladonema radiatum*, another creeping form, is also very variable, the number of oral lobes being five or seven, and that of the tentacles and canals eight or ten (Hincks).

The facts of Echinoderm and Cœlenterate anatomy above quoted, suffice to illustrate the statement that in animals whose organs are already radially repeated, variations consisting in the repetition of one or more of the peripheral organs is of common occurrence, and may affect large numbers

[1] In relation to this acquisition of the appearance of longitudinal repetition or segmentation by a radiate animal, an example of the inverse phenomenon may be given. Among the Operculate Cirripedes, though in the Balanidæ the arrangement of the six plates composing the "cone" are so placed as plainly to indicate the original long axis, yet in the Coronulidæ this feature becomes obliterated, and the plates are disposed in a *radially* symmetrical manner.

of organs as in the case of the arms of Asteroidea, and may be of specific occurrence as in Asterias rubens and Brisinga coronata, or even ontogenetic as in Clavatella, &c.

All the instances of repetition of organs which have been so far selected, whether in the case of animals with a marked long axis or in the radiate forms, have been examples of the recurrence of parts or organs in some more or less definite relation to the axis of symmetry of the animals. These have been chosen especially as more markedly illustrating the possibility that the segmentation of some forms at all events may have been derived from the continual recurrence of this phenomenon until it became more or less regular and transmissible to the offspring as the definite course of development. But it must be remembered that repetitions of this kind are of an extreme type. The recurrence of whole sets of organs, as in the case of the arms of Asterias or the gastric pouches and generative organs of the Nemertines, must be regarded as the higher manifestations of this phenomenon, and consequently of more or less occasional occurrence. Since, however, it is in these cases that the nearest approach has been made to metameric segmentation as we now see it, they have necessarily been selected as of the first importance. But if repetitions of this magnitude are of rare occurrence, repetitions of smaller parts or organs are extremely common, if not universal. There is hardly one of the larger or more organised types in which whole tracts of the body are not composed of almost precisely similar and "serially homologous" parts, which are of very variable number. The scales and fin-rays of fishes, the tufts of hair and markings on many caterpillars, the teeth of Vertebrata, the joints of the Arthropod appendages, or of the stems of a Crinoid, the ossifications in the ambulacra of the Echinodermata, and many others, suggest themselves at once.

Especially noticeable are the casual repetition of large complex structures, such as the mammary glands and of exoskeletal organs, as the horns and dermal scutes of Vertebrates. The

number of these is liable to great variations, not even being constant in the species. For example, certain deer and also certain sheep have specifically more horns than two; and in the case of Iceland sheep the horns may be three, four, or five (Youatt, 'The Sheep'). By the nature of the case none of these repetitions can be atavistic; and it is interesting to notice how, just as it was shown that irregular repetitions of parts about the axes of symmetry of the body often take up regular secondary relations to them, recurring either in segmental pairs or in radial symmetry, so these minor repetitions take up regular relations (secondary in some cases, probably primitive in others) to the axes of the limb or part of the body in which they occur. Thus the ossifications in the Crinoid stem or the Starfish arm are so regularly related to the axis of the part that in the latter case they have suggested to Haeckel his extraordinary view of the phylogeny of the group, appearing to him precisely similar to the segmentation of a Chætopod. The case of the scales of fishes and the hairs and markings of caterpillars should perhaps have been more properly quoted in the former connection, as being an instance of irregular repetitions which have become definitely related to the symmetry, as in the case of the Sturgeon, and among caterpillars the Tussocks and the Spherigidæ. One very curious instance may be quoted of a series of repetitions which, though essentially arranged with reference to the axis of a limb, have yet a definite relation to the long axis of the body. This instance is that of the Vertebrate tail, which has often been adduced by opponents of the Annelid theory of Vertebrate descent. Now, the structures which repeat themselves in the Vertebrate tail with great variability of number, namely, the vertebræ with their neural and hæmal arches, the segmental vessels and nerves, &c., are precisely those structures upon whose repetition in the trunk the view of the primitive character of the segmentation of the Vertebrata mainly depends.

In the foregoing pages the attempt has been made to show that greater or less repetition of various structures is one of the chief factors in the composition of animal forms, that these

repetitions may be of greater or less extent, affecting single or many organs, and may be at first irregular, and finally culminate in regularity, and that even this regularity may afterwards vary so as to become a symmetry of a different order. It is further contended that between repetitions in these varying degrees it is impossible to draw any hard and fast distinction, for nothing more can be affirmed as yet about them than that they are repetitions. The reason for their appearance is as yet unknown, and the laws that control and modify them are utterly obscure. But in view of what has been adduced it is surely not too much to say that enough of their mode of working can be seen to enable us to realise that they are at least powerful enough to have produced anatomical features of high importance, and further that the metameric segmentation of the Vertebrata is distinctly of the kind which could be brought about by their operation. That in this case they have attained a degree of completeness far exceeding that which they elsewhere present must be admitted ; but there is no evidence to show that this result differs in kind from that which occurs on a smaller and more restricted scale in almost all animals. Whether the repetitions which occur in the Annelids and Arthropoda are also the products of this force in a still higher degree cannot yet be certainly stated.

General Conclusions as to the Mode of Occurrence of Repetitions of Organs.

In the present state of biological knowledge no guess can be hazarded as to the cause of the facts above quoted. The solution of the problem must be sought in a fuller knowledge of the laws of growth and variation, of which we are still ignorant. As yet only one or two features in these repetitions may be mentioned as possibly of importance, though even these can only be selected in the most tentative manner.

In this connection the first noticeable fact is that the structures repeated in the Triploblastica are very generally of mesoblastic origin, and that when other structures have become involved this would appear often to be a secondary

occurrence. To such an extent is this true that in a recent contribution to this subject (Caldwell, 'Quart. Journ. Mic. Sci.,' 1885), a suggestion has been made which proposes to give a simple physical explanation of all the phenomena of segmentation. Caldwell suggests that owing to the early acquisition of the long axis of the body and the consequent elongation of the blastopore, the mesoblast has become, so to speak, left behind in blocks, in consequence of the more rapid growth of the epiblast. That this extremely simple theory will not account for all cases of repetition is shown, firstly, by the fact that though the repeated structures are generally mesoblastic, yet they are not always so; secondly, that the mesoblast does not thus originally segment as a whole, but rather that separate organs repeat themselves separately, as has been already urged, especially in the case of the Turbellaria; and finally, these repetitions are by no means universally embryonic or even larval features, but their whole history rather points to their having very generally originated in the adult condition, and to the view that they have come to be thus earlier in development, the opposite of which is assumed by such a hypothesis as Caldwell's.

This belief that these repetitions have had their origin in variations which occurred in the first instance late in life is founded upon several considerations. Firstly, the cases in which the generative organs are repeated are very numerous; in fact, both organs or the testis, at all events, are repeated in nearly all the cases in which much repetition is found (in most Dendrocœles, Chætopods, Nemertines, Balanoglossus, Amphioxus), even if few other systems are repeated. In the case of these organs it is most likely that the repetition first arose in adult life, and, in fact, in most of them it does still so arise; that is to say, the masses of cells which are to form generative organs are not specially broken up at an early age. And in the second place, the original late origin of repetitions is likely from the fact that most of them still so arise; it is only in exceptional cases as that of the mesoblastic pouches of Vertebrata, Phoronis, Enteropneusta, and the horns of the

water-vessel of Echinodermata, that some of the repetitions are presented early in the development.

Besides the probability that most repetitions occur in the first instance in adults, or, at least, in mature individuals, it may also be noted as a general feature of them that they are at first very similar to, if not identical with, each other. For on their first appearance in an individual they do not generally arise phylogenetically in the condition which may be supposed to have been that in which the original organs of the same series first arose, but rather from the first they are found as fully differentiated copies of the other members of the series, and not as rudiments. For example, the horns and teeth of mammals, whose number varies greatly, are, in those forms which possess additional ones, not repeated as tubercles or as plates, but rather as fully developed horns, teeth, &c. Though this is not universally true it is yet sufficiently well marked a feature to be of great importance in estimating the probability of the recurrence of such a complicated organ as a vertebra with its correlated parts within narrow limits of race. But no less noticeable is the tendency towards a subsequent differentiation and division of function among members of a series of similar parts as soon as the series is formed or any new member is added to it. This is of course to be seen in the case of the tentacles of Hydromedusæ, the division of the ambulacra of Echinoderms into bivium and trivium culminating in the bilateral symmetry of Holothurians, differentiation between vertebræ, &c.

Beyond this little can be predicated of the mode of occurrence of repetition of parts. Nothing is attained by analysis of the known facts which can be felt to be in any way a basis from which to interpret them. This much alone is clear, that the meaning of cases of complex repetition will not be found in the search for an ancestral form, which, itself presenting this same character, may be twisted into a representation of its supposed descendant. Such forms there may be, but in finding them the real problem is not even resolved a single stage; for from whence was their repetition derived? The

answer to this question can only come in a fuller understanding of the laws of growth and of variation which are as yet merely terms.

Preliminary Remarks on the Repetition of Organs of the Chordata.

In the foregoing pages it has been attempted to show (1) that repetition of organs and sets of organs is of common occurrence among animals, and (2) that however far back a segmented ancestor of a segmented descendant may possibly be found, yet ultimately the form has still to be sought for in which these repetitions had their origin. Hence it follows that in no case must it be held *à priori* impossible that an unsegmented form showing no degeneration should be related to a segmented stock. But when inquiry is made in the special case of the Chordata as to the condition of the repetitions found among them, it will be seen that so far are they from suggesting that their immediate ancestor of the group must have been segmented, that they even preclude this view. As will be shown, there is a history of the actual steps by which several of the organs (the nervous system, the axial skeleton, and the mesoblast) acquired their repetitions within the group, and certain other structures (the notochord, &c.) persist in an unsegmented form. So that instead of regarding a fully segmented form as their possible ancestor it is necessary to search for a form in which these particular sets of structures at least are not repeated.

For in the first place, taken generally, the development of a Vertebrate consists in the gradual appearance of repetitions, first of one organ and then of another, until at last a climax is reached. The mesoblast divides into blocks, paired peripheral nerves grow out, and segmented tubules arise in connection with the excretory ducts, but the mesoblastic plates were at first unbroken, the medullary plate continues without transverse divisions, though its peripheral organs may be repeated, and the excretory ducts are single tubes with single openings. That many of these structures roughly correspond with each other

is no doubt true, but these correspondences are only partial, and, as will be shown in the sections on the nervous system and vertebral column, a history is preserved to us of the steps by which some, at least, of these repetitions have been attained and of stages in which these correspondences were still more irregular.

The attempt to find the ancestor of the Chordata resolves itself first into the question as to whether the Chordate features, viz. notochord, gill-slits, and nervous system of a particular type were first associated in a form which possessed repetitions in a high degree or not. Now, since the notochord is always unsegmented, it is à priori likely that it arose in an unsegmented form; for, having in view the early period of development at which it arises and the situation which it occupies in the body, and the fact that it is found in the dorsal wall of the gut, the sacculation of which is one of the commonest features in segmented forms, it could hardly have thus arisen without participation in such segmention. On the hypothesis of Annelid descent the facts of the morphology of the notochord are inexplicable; for, seeing that no homologue of the notochord exists among Annelids, on the theory that Vertebrates are their descendants, the notochord must have arisen subsequently to that segmentation, to account for which the Annelid ancestor is postulated. If this were so the notochord, by every rule of phylogenetic interpretation, might be expected to arise late in development, and to exhibit marked segmentation, instead of which it is almost the earliest organ formed, and is absolutely unsegmented.

Similarly from the first, the medullary plate is distinctly a single structure, and without suggestion of transverse division. Not until the peripheral nerves arise is any serial repetition to be found in it, and were it not for theoretical considerations it would not have been supposed that the nervous system of a two-day Chick was a segmented structure. Further, in Amphioxus and the Marsipobranchs the serial repetition, even of the peripheral nerves, is not regular and opposite, the further meaning of which facts will be discussed later.

Lastly, the gill-slits are by their nature repeated structures; but, seeing that nothing resembling them occurs outside the group,[1] their origin and, à fortiori, their repetition has been acquired within it.

It becomes then probable, from preliminary examination of the morphology of the three typically Chordate features, that their first origin was not in a segmented form. There is also one other structure which certainly points in the direction of an unsegmented animal as the immediate ancestor of the Vertebrate. This structure is the liver. Now, the liver is essentially a unique structure in the body which is not repeated. On the Annelid theory of Vertebrate descent it would have to be supposed that the liver either arose as an enlargement of one of the segmental saccules of the gut, or by the coalescence of several. The evidence attainable on this point is distinctly against either of these possibilities; for the liver of all the Vertebrates, and especially of Amphioxus, is markedly and obviously a single structure, not formed by the coalescence of several, while its asymmetrical position and general appearance favour the view that it is a structure newly formed within the limits of the group, rather than a relic of a paired sacculation.

Having then disposed of the à priori odjections to regarding an unsegmented form as a primitive member of the group, the attempt will be made to show that the Eteropneusta occupy this position. After this we will proceed to consider the light which this admission will give on the history of the steps by which the organs of the other Chordata acquired their present arrangement, and finally to determine the relation which the various forms included under this head bear to one another.

The Enteropneusta as Members of the Chordata.

The general features of the anatomy of the Enteropneusta place them in a very isolated position. They are extremely

[1] For Semper's suggestion that the cœlomic pores on the heads of some Oligochæts are of the same nature cannot be seriously considered.

like one another, but apparently very unlike any other group of animals. Before Tornaria was known to be a stage in their development they were assumed to be worms of some kind, but after Metschnikoff had succeeded in proving Tornaria to be the larva of a Balanoglossus this was felt as an impossible view of its affinities. Up to this time Tornaria had been regarded by Joh. Müller, who first described it ('Berl. Akad.,' 1849, 1850), and by others who examined it as a varied form of Bipinnaria, which, indeed, it very closely resembles, differing only in the presence of eye-spots, and of a peri-anal ring of cilia; both of which structures are liable to great variation. When, then, Metschnikoff discovered its real destiny, it appeared at first sight necessary to suppose the Enteropneusta closely connected with the Echinodermata, and accordingly Metschnikoff ('Zool. Anz.,' 1880) proposed to include them in a division Bilateralia under the Echinodermata, the remainder of the group forming a parallel division, Radiata. But this generalisation with regard to the group was made solely on the characters of the larva, and almost without reference to the structure of the adult, which, indeed, was little known. So certain, however, did the conclusion seem, that Metschnikoff was led to suppose that the gill-slits of Balanoglossus were mere amplifications of the water-vascular system of Echinoderms, which could hardly have been suggested had it not been felt that no other solution was possible. Since this time the anatomy of the adult has become more fully known, and another mode of development has been shown to occur, and from neither of these additional sets of facts can any confirmation of the Echinoderm theory be derived. Hence we must conclude that the characters of Tornaria are not to be looked to solely in attempting a solution of the problem.

In the development of Balanoglossus Kowalevskii the following important features occur: (1) the origin of the central nervous system is by longitudinal delamination from the skin in the dorsal middle line; (2) at the anterior end of the body a portion of hypoblast is constricted off on the dorsal side to form a supporting structure, i. e. a notochord; (3) the

gill-slits are formed as regular fusions and perforations of the body wall and gut from before backwards. Hence the three features which alone distinguish Chordata from other animals are present, and associated from an early period in development. Added to this the minor features of Chordate anatomy are also represented by (1) the origin of the mesoblast; (2) the remarkable asymmetry of the anterior parts; (3) the opercular fold; (4) the excretory funnels opening into the atrial cavity thus formed. From all these facts we may form a preliminary conclusion that the Enteropneusta bear some relation to the Chordata. We will now discuss what relation this is, and before doing so we must determine what relative importance is to be attributed to the two modes of development known to occur, the one largely embryonic the other pelagic.

In our present state of ignorance as to the mode of development of Tornaria and of the details of its later stages, it is difficult to compare these two modes, but the question as to which is to be regarded as primitive is probably a part of the larger question as to the comparative likelihood of the preservation of ancestral features in the free or in the protected developments. This question cannot be fully gone into here. No general answer has as yet been given to it, and since the balance of probability is very nearly divided between these two possibilities we may be right in assuming either of them to be correct. For the purposes of the following argument it will be assumed that, on the whole, development within an egg-shell, as involving a less complicated struggle with environmental forces, is less subject to variation than that in the open sea, and consequently is more likely to preserve ancestral features. Besides this, in the special case before us, the adult structure is practically conclusive against Echinoderm affinities, to which the pelagic development would point if regarded as primitive.

Assuming, then, that the development of B. Kowalevskii is more primitive than that involving a Tornaria stage, the following features are of great importance:

(1) The animal is ciliated and inhabits muddy sand.

(2) The præoral lobe is enormously developed.

(3) The notochord arises at the anterior end of the hypoblast and grows forwards.

(4) The origin of the central nervous system consists in the delamination of a solid cord of epiblast in the dorsal middle line of the middle third; this, by invagination of its two ends, is afterwards extended as a tube in both directions.

Other collections of nerve-fibre are afterwards deposited in various parts of the body, and finally a general network of nerve-fibre occurs at the base of all the skin of the body, especially in the line of the gill-slits.

(5) The mouth originally faces ventralwards, but comes afterwards to open forwards, being not a sucking but a digging mouth.

(6) The gill-slits for a long time are only one pair, but subsequently are repeated in pairs, increasing in number with increase in the size of the body.

(7) The mesoblast arises as one unpaired pouch, followed by two pairs of pouches.

(8) The blood-system is entirely peculiar, consisting of an anterior heart and a dorsal and ventral vessel, and in B. minutus of two lateral vessels in the intestinal region. The two former are united by a plexus of trunks, which are placed under the skin and below the walls of the gut.

(9) The generative organs are repeated through a large part of the body; in the branchial region more or less following the repetition of the gill-slits.

(10) Of the excretory system little can be affirmed. The cells of the mesoblast appear to have a power of forming concretions, probably excretory, in their substance, and then throwing them into the body cavity. Here they form small aggregations. A large gland (containing a plexus of vessels), apparently performing their function, exists in the proboscis cavity attached to the end of the notochord.

From the proboscis cavity opens an asymmetrical ciliated

pore, placed on the left side of the body, which in B. Kupfferi is stated to be paired.

From the middle body cavities open a pair of pores into the atrial cavity, which is partly enclosed by

(11) A rudimentary operculum.

Having these facts in view, and having set aside the preliminary objection that no high degree of segmentation is present in Balanoglossus, we may consider their bearing on theories as to the ancestry of the Chordata.

Previous Suggestions as to the Ancestry of the Chordata.

Setting aside the possibility of Annelids having been genetically connected with the Chordata, the most notable alternative suggestion is that of Balfour, that the Nemertines might be thus regarded. This view has been supported and extended by Hubrecht. It has thus been thought that the Chordate nervous system might have arisen by the longitudinal coalescence of two such cords as are present in Nemertines. But even the facts of other Chordate developments almost preclude the view that their nervous system is a double structure; the medullary plate of Amphioxus is distinctly single, and it is only in the medullary folds of higher and more complex forms that even an appearance of a double structure is produced, while no really double origin occurs. This being so, the mode of origin in Balanoglossus is practically conclusive against the theory of double origin. It is possible, and even likely, that Nemertines bear some distant relation to Chordata, as will be further discussed subsequently, but if this is so it can no longer be supposed that their nervous system is other than a special development within the group.

In most speculations as to the origin of Vertebrata, it is assumed that all the lower forms of Chordata are degenerate. The supporters of the Annelid theory especially are compelled to resort to this view severally in the case of the Ascidians Amphioxus, and the Marsipobranchs. These, with the exception of the Enteropneusta, are the only forms which could have

been used to throw light on the origin of the group, and they had to be expressly excluded because the suggestion as to the origin of the group had been made without regard to them. In the case of Amphioxus and the Marsipobranchs this theory of degeneracy will not bear examination.

It rests solely in the one case on the fact that Amphioxus has no developed sense organs and lives buried in the sand, and in the other on the semi-parasitic habit of life of the group. This degeneration is postulated to explain the lower degree of segmentation presented by these forms; and the fact remains that of all animals the worms which live most underground are the most segmented types which are known. Hence it cannot be assumed without ontogenetic evidence that degeneration in this direction has occurred. This ontogenetic evidence is entirely absent. Degeneration in this sense means a phylogenetic change of plan; and this change of plan should then leave a mark on the ontogeny, as occurs in Echiurus, &c.; but no event in the development of Amphioxus or of Lampreys points to any such change of plan. The development of these forms is a steady progress up to the point which the creatures finally reach, and in a case of this kind it is gratuitous to postulate degeneration in order to support a preconceived view of the morphology of the group. (Even in the Ascidians, though a well-marked change of this kind does occur, yet it is not a deviation from a segmented to a less segmented form; for with the doubtful exception of Appendicularia, Ascidian tadpoles are quite without trace of segmentation.)

Again, no such evidence of a change of phylogenetic plan is found in the case of the Enteropneusta. Highly modified, no doubt, the adult animals are, but not degenerate. For these reasons the presumption of universal degeneracy on the part of all the lower Chordata will be dismissed, and an attempt made to systematize the facts as they are found.

The Habits of Life and Form of the Body of the Primitive Chordata.

Habits of Life.—The presence of gill-slits in all the Chordata may be taken as positive evidence that they arose in an aquatic habitat. Moreover, such a structure as the notochord cannot be conceived as having arisen in a fixed form. Hence they probably led a more or less free existence. This being so, they may either have been pelagic creatures, as the larvæ of Amphioxus, or may have crept in mud as the larvæ of B. Kowalevskii. Between these two possibilities there is little or no determining evidence. The only feature which seems likely to affect the question is the question as to the original point in the body at which the notochord first segregated itself from the gut. Unfortunately the evidence upon this point is divided. For if we suppose that the condition in Balanoglossus is primitive, and that notochord began as a rod in the dorsal wall of the anterior end of the hypoblast, then this origin would more or less point to a burrowing habit, the notochord functioning as a support for the head in this operation; but if the separation of the notochord in the middle of the body, as in Amphioxus, be held to be primitive, then this would point to a pelagic habit, the notochord serving as a fulcrum, from which the movements of the animal in swimming might be maintained. The absence of fins on the young Balanoglossus and on the young Amphioxus, though pelagic, appears to point slightly in favour of a burrowing habit, though no reliance can be placed on such slight negative features.

Primitive Mouth.—There is one more point that does point in favour of a pelagic habit, namely, the fact that the anteriorly-directed digging mouth of both Balanoglossus and of Amphioxus is of secondary origin, being formed by a modification of a more primitive ventrally-directed mouth.

Balfour, having the mouth of Lampreys and Tadpoles in view, held that the original Vertebrate mouth was suctorial. This the ventrally-directed mouth might have been; but this

fact does not interfere with the obvious possibility of a digging mouth having again intervened, from which such a mouth as that of the Lampreys could easily be derived.

Taking into consideration, then, the fact that in the most primitive forms the mouth is anteriorly directed, and that in the Lampreys it is also anteriorly directed, though of different function, we may tentatively suppose that though the mouth of the possibly original pelagic form was directed ventralwards, and was possibly suctorial, yet probably the mouth of the Marsipobranchs is derived from a digging ancestor, in which the mouth of the hypothetical pelagic form had come to be anteriorly directed in correlation with an acquired burrowing habit. In any case the facts of the Enteropneusta entirely confirm Balfour's view, that the Vertebrate jaws have been developed comparatively long afterwards.

The Skin.—That the skin was originally ciliated there can be little doubt; also it is probable that at first plexuses of nerve-fibre were formed at the base of the ectoderm cells, such as may be seen in many if not in all animals with ciliated skins of this type.

The Nervous System.—The next question relates to the position and mode of the first formation of a differentiated nervous system. The evidence of Enteropneusta, Ascidians, and Amphioxus is united in showing that this first occurred in the dorsal middle line, and not by the coalescence of two lateral cords. The structure of the nervous system of Balanoglossus further shows us a stage in the process by which this nervous cord separated from the skin. By many authors it is supposed that this was accomplished in the first Chordata by an invagination, but the evidence of Balanoglossus is decidedly for the view that a process of delamination preceded this; and, indeed, this being the simple process, might naturally have been expected to have occurred first. In Balanoglossus we see in the trunk the cord still in the skin, in the collar the cord delaminated, and at the ends of this cord the process of invagination commencing and leading to the presence of a lumen. More than this, the mode of

origin of the peripheral nerves is also seen; for those portions of nervous tissue which remain in the skin consist of fibres and a few cells. Into the nervous tissue thus composed run the tails of ectoderm cells, and out of them, on their inner sides, run many fibres into the subjacent mesoblastic tissues. Now, the fibres entering this nerve-substance on its outer side are plainly sensory, or at all events afferent, and the fibres passing from it on its inner side are presumably motor, or at least efferent, seeing that they innervate the mesoblast.

It is clear, then, that on the separation from the skin of a cord thus composed the relations of the efferent fibres will not be changed, as they still remain in contact with the mesoblast. But, on the other hand, if this nerve-cord be entirely separated from the skin the supply of outer or afferent fibres is cut off from it, unless cords of epiblast remain to connect it with the skin. Applying this reasoning to the particular case of the separation of the dorsal cord, we see that the afferent fibres are entering it on its dorsal side, and that the efferent fibres are leaving it on its ventral side. If, then, the cord sinks in from the skin, the efferent fibres coming out on the ventral side to supply the muscles can still do so without being gathered into cords, remaining irregular as they do in Balanoglossus, but without dorsal cords connecting the main cord with the skin afferent impulses could only enter at the two ends which remain connected with the skin; hence I submit that it is probable that the three median cords in Balanoglossus minutus, &c., are to be regarded as the homologues of the dorsal roots of other Chordata. It is at once evident, from the physical exigencies of the case, that if the nervous system arose in this way the dorsal roots were from the first sensory, and that they did not arise as differentiations of roots of mixed function, as has often been supposed. If this is true, then, as the cord phylogenetically comes away from the skin from before backwards the number of these dorsal cords will increase, until finally the cord lies connected all along the body with the skin by a series of median dorsal cords placed at intervals.

Now, returning to what is found in Balanoglossus, it is to be noted that, first, the cord separates from the skin as a solid rod connected at the two ends to the skin, and upon this condition invagination supervenes at the two ends, forming a neural tube in these regions. Let us follow the effect which an extension of this system of invagination along the cord will have upon the origin of the dorsal roots; for it is nearly certain that invagination in this case is secondary to delamination; the condition in Amphioxus, in which the medullary plate folds up after being enclosed, offering a stage of transition between the condition found in Balanoglossus and that of an Elasmobranch, for example. Since the invagination of a plate of tissue differs from the separation of a cord in the fact that it is not the central line, but the two edges of the plate, which remain last in connection with the skin, it follows that, as the process of invagination phylogenetically arrives at the point of attachment of any one of these median dorsal roots, it must take up its new attachment at one of these two edges. It is thus not possible, supposing these views correct, that the dorsal roots could in the first instance have been paired, except on the hypothesis that as the process of invagination phylogenetically reached its point of attachment each dorsal root split into two; which is almost impossible, and which the condition of Amphioxus shows not to have occurred. The other alternatives would be (1) that all the dorsal roots should remain attached on one side to the cord; (2) that they should be attached irregularly to one side or the other; and lastly (3) that they should have been attached alternately to either side. From the nature of the case they could not be opposite. Now, the fact of their alternate arrangement in Amphioxus is almost a proof that the latter alternative was the one which occurred. (It may be observed that, as a physiological convenience, they probably supplied the two sides of the body alternately while yet attached in the middle line.) Thus the opposite origin of the dorsal roots is almost certainly secondary to an alternate arrangement. The fact that it is the foremost pairs which are opposite in

Amphioxus seems to indicate that the process by which they became so occurred first anteriorly.

Let us now follow the history of the ventral roots as preserved to us. In Amphioxus the large nerves or dorsal roots supply the skin and certain sense organs placed among the muscular tissue (Rohon); but into each myotome, opposite each dorsal root, runs a bunch of loose nerve-fibres from the cord. This was stated by Rohon, but denied by Balfour. Improved methods of section cutting leave no doubt, however, that Rohon's observation was correct, and, indeed, these fibres may be easily seen. The presence of these bunches of fibres clearly gives us another step in the formation of the "segmented" nervous system. For in the simplest case, that of Balanoglossus, the muscles are not gathered into bunches, and the nerve-fibres likewise are irregular. In Amphioxus the muscles are already gathered into bundles, and the motor nerves follow them in this arrangement, but remain distinct from the dorsal roots. This therefore is a stage towards the gathering of the efferent fibres into a "ventral root;" in Bdellostoma this is already done, and though the dorsal roots are already approximately, though not quite opposite each other, yet the ventral roots are not at the same level with them. Besides this, in Lampreys, the anterior and posterior roots are still not united into a common cord, though in Myxine they are thus arranged (Schneider and others).

In this the nervous systems of Balanoglossus, Amphioxus, Lampreys, and Myxine form a graduated series leading up to the condition found in higher Vertebrates, showing the evolution of the nervous system of Vertebrata from a solid cord in the skin to its condition as a closed tube whose walls give off a series of "segmental" nerves arising by roots of different functions.

[It will be seen that if this view be accepted it becomes very doubtful whether efforts to analyse the segmentation of the head can lead to any result, seeing that it almost follows that the head was differentiated as such before any complex metamerisation was present; and, indeed, were it not for theoretical

considerations, it could hardly have been supposed that the head of a three-day chick, for example, was a highly segmented structure, seeing that the regular segmentation of the body conspicuously stops at its junction with the trunk. No doubt the cranial nerves may, by arbitrary divisions and combinations, be shaped into an arrangement which more or less simulates that which is supposed by some to have been present in the rest of the body, but little is gained by this exercise beyond the production of a false symmetry.]

The Axial Skeleton.—The notochord of the Enteropneusta is so partially developed that it is not difficult to conceive that its presence in the middle third of the body may indicate a stage in its phylogenetic appearance. If while in this condition it was used as a fulcrum in swimming it seems further conceivable that if this organ grew backwards the condition of the Ascidian Tadpole's tail would be produced, though no stress can be laid on this view. As will be shown later on, it is likely for other reasons that the Ascidians separated themselves from the other Chordata before Amphioxus, or even the Enteropneusta.

By extending the separation of the notochord the condition of Amphioxus is reached. And next, the axial column of the Marsipobranchs shows us the notochord enclosed in a mesoblastic sheath as yet unsegmented. This process is foreshadowed by the presence of rings round the neural canal, placed between the nerves whose segmentation they follow. Finally, in the other Vertebrata the column itself is segmented, so that this is another instance of the appearance of a typical segmentation in a system of a Vertebrate whose origin within the limits of the group is unmistakeably traceable.

The Myotomes.—Intermediate conditions between the condition of the muscles of Balanoglossus and of Amphioxus are as yet unknown. I submit, however, that it is not impossible to conceive the formation of Myotomes by a simple mechanical process of gathering the muscular fibres into bundles. Their origin as Archenteric pouches may then be supposed to have originated from the fact that the ancestral

mesoblast already arose thus, and when new bundles of muscles formed in the adult began to arise in the larva they arose in the same manner as the primitive mesoblast. That provision is made for the production of more mesoblast than that of the original fourteen pairs of pouches is shown by the presence of mesoblastic pole-cells in Amphioxus (Hatschek). In any case the existence of Balanoglossus proves that the notochord, gill-slits, and Chordate nervous system were present together before the myotomes were formed.

The Gill-slits.—It is unfortunate that the facts of the Enteropneusta seem to throw no new light on the original meaning of gill-slits. That they do not do so tends, however, to show that probably gill-slits were from the first developed as such, and not as modifications of any previously-existing organ, as has been sometimes held.

The folded skeletons of the gill-slits of Balanoglossus are remarkable in their resemblance to those of Amphioxus. Until the development of these latter is fully known no further comparison can be instituted. It is clear from their origin in Balanoglossus that no "myotomes" are obliterated between them (as has been suggested by some, with the hope of increasing the symmetry of the body), for plainly their repetition preceded that of the myotomes.

The Excretory System.

Upon the origin of the excretory system of Vertebrata nothing can be affirmed from a study of Balanoglossus. The excretory systems of Vertebrata cannot be easily derivable from anything found in either Balanoglossus, Ascidians, or Amphioxus. The absence of any regular excretory system in these three forms may, perhaps, be correlated with the extraordinary development of their respiratory systems, which may possibly assist in this function. The one fact which is derivable from the morphology of Balanoglossus, Ascidians, and Amphioxus, is that it is nearly certain that the excretory system of other Chordata has been developed within the group.

The Pituitary Body and Proboscis Pore.—Though

no insistance is placed on the following suggestion, the plausibility of it is such that it cannot be omitted. On a previous occasion I have called attention to the fact that the pore which in Amphioxus leads into the left anterior body cavity is obviously homologous with the proboscis pore of Balanoglossus, which leads from the left horn of the anterior body cavity. In some species of Balanoglossus the opening of this pore is placed medianly, though opening into the left horn. Now, supposing the præoral lobe to atrophy, as in an Ascidian, so that the neural pore came to open into the buccal cavity, as occurs in these forms, it is clear that any pore placed dorsally between the neural pore and the mouth will then be directed ventrally, and open into the pharynx below the end of the nervous system. This is precisely the position occupied by the ciliated pit of an Ascidian, which leads into the gland described by Julin ('Arch. de Biol.,' 59). Hence with this pore and gland of an Ascidian the proboscis pore and gland of Balanoglossus may be compared. Next, supposing the end of the nervous system to dilate and form a brain which bends up by a cranial flexure it follows that on the atrophy of the proboscis (or rather before the proboscis was formed, this being peculiar to Enteropneusta) this pore will lie in the dorsal wall of the stomodæum, i. e. in the position of the pituitary body. More than this, any gland attached, as is the proboscis gland, to the end of the notochord, will, when this is flexed by the cranial flexure, be bent backwards with it to the place where its end comes to lie, i. e. above the pituitary involution. In this way the double structure of the pituitary body becomes intelligible. If these views are correct the pituitary body and its pore is to be regarded as the rudiment of a primitive excretory organ, which originally opened dorsally.

I have elsewhere shown the *primâ facie* resemblance of the anterior body cavity with its pore in Amphioxus to that of Balanoglossus, which in the Tornaria development is formed from the water-vessel (Spengel). This water-vessel is precisely similar to that of Echinoderms, being otherwise without parallel among animals.

The Affinities of the Chordata.

Having thus examined the history of those organs which the morphology of Balanoglossus enables us to trace, let us consider the relations of Chordata (1) to other groups, (2) to each other.

Of the Echinodermata.—Unlikely though it may seem, if any reliance can be placed on the characters of pelagic larvæ, we must assume some affinity between Echinodermata and Chordata, for Tornaria is not very like, but practically identical with, Bipinnaria. The case is like that of Mollusca, which may be supposed to be allied to Annelids, as is indicated by the trochosphere larva.

Of the Nemertines.—So much has been said by previous writers as to the Chordate affinities of Nemertines that the subject cannot be omitted. The suggested homology of the nervous system has already been dismissed. Hubrecht has further suggested (1) that the notochord is homologous with the proboscis sheath of Nemertines, (2) that the cephalic pits are gill-slits, (3) that the proboscis is the pituitary body.

With regard to (1), what can be adduced from a study of Enteropneusta seems rather to be opposed to this view. If this were true, the notochord must have arisen in some such body as that of a Rhabdocœl, into the wall of the endoderm of which a præoral lobe could be invaginated, rather than as a hard thickening which is constricted off to form a lumen. Into the free end of such a structure it is impossible to conceive the invagination of a proboscis, which is what Hubrecht's suggestion seems to require. All that can be said is that the notochord of Balanoglossus suggests that it arose as a supporting structure and not as a modification of something else.

But supposing the larva in Stage G to represent a phylogenetic phase, several points of Nemertine anatomy can be derived from it. At this stage it has one pair of gill-slits, a short nerve-cord, one median anterior mesoblastic pouch, and two pairs of posterior pouches. Now, on the hypothesis of

Hubrecht that the œsophageal pouches of Nemertine were the homologies of gill-slits, and supposing the proboscis invaginated and around its base a quantity of nerve-tissue deposited as in Balanoglossus, the proboscis would then have the same relation to the nerve-ring as that found in Nemertines. Hubrecht's view of the pituitary body falls if the alternative here given is accepted. Though the points of anatomical resemblance are not striking, yet when taken with the ciliated skin, the ventral mouth and position of the generative organs they form a basis for comparison.

If these resemblances were found to be real the nervous system of the Nemertines would have to be supposed to have arisen within the limits of the group. As both animals possess a nerve-plexus in the skin this does not seem impossible. Also the excretory system lately described by Oudemans ('Quart. Jour. Mic. Sci.,' 1885), would have thus arisen as a specialization of parts of the body cavity; since in Balanoglossus this function appears to be generally distributed over the body cavity, this also might be conceived.

Of the Tunicata.—Next, since all the Chordata at some period of their development agree with the larva in Stage H, in possessing a dorsal nerve-cord more or less invaginated, one or more pairs of gill-slits and a notochord, let us pass on to Stage H, in which the notochord is forming at the anterior end of the gut. From such an animal as this the Ascidians may have been descended. For, as has been suggested by van Beneden and Julin ('Archives de Biologie,' 1885) it may be, that all the Ascidians have but a single pair of gill-slits; for that Appendicularia has only one pair is known; while in some genera the atrial cavity arises as an increase in the size of the pair of ciliated chambers by which the gill-slits open; and this increase may take place in the hypoblastic half of the chambers, or in the epiblastic; by the fusion of these two chambers the atrial chamber of these genera is formed. Van Beneden and Julin then suggest that the atrial pore is the actual opening of the two fused gill-slits, and that the rows of slits placing the pharynx in communication with the atrial

chamber are to be regarded as secondary perforations. Whether this ingenious theory be adopted or not, the fact remains that Appendicularia is almost certainly a very primitive Tunicate, and also that the arrangement of the pharyngeal perforations of other Ascidians makes it unlikely that they are homologous with the gill-slits of higher forms.

The increase in size of the tail, which would speedily follow the first use of the backward directed notochord as a swimming organ is not difficult to understand. In connection with the increase of the tail the curvature of the gut would also be intelligible. From atrophy of the præoral lobe in correlation with the future sessile habit, coupled with increase of the lower lip to bear the suckers, the relations of the neural pore to the mouth would result. The gland of the præoral lobe would then, as before described, be placed below the nerve-ganglion and open into the pharynx.

It has been remarked by Seeliger (' Jen. Zeit.,' 1885) that the body of the Ascidian tadpole appears to consist of one head and two trunk segments. It may be observed that though the reasons for this belief are not very obvious, this view, if correct, would coincide with the possibility of its descent from such a larva as Balanoglossus, Stage G, which also possesses one head and two trunk segments.

However the various points that have been raised in the preceding paragraph may be decided, it has seemed necessary to point out what conclusion with regard to the structure of Ascidians may be drawn from the development of Balanoglossus. That these are so meagre is to be regretted; the only tangible point appears to be the confirmatory evidence that it offers to the view that the atrial folds of Tunicata are not homologous with those of Amphioxus.

In this way only can the absence of mesoblastic repetitions in Tunicates be accounted for. Their development gives no support to the view that their ancestors possessed repetitions of this kind.

Of the Enteropneusta.—That the Enteropneusta might possibly have had an ancestor in an animal possessing the

structure of Stage H is of course shown by their ontogeny. They are derived from it chiefly by increase in size of the præoral lobe, change in direction of the mouth, growth of a rudimentary operculum, serial repetition of the gill-slits, and appearance of the generative organs also as a serial repetition. That any animal possessing a large præoral lobe should acquire a thick sheath of nervous tissue (especially when consisting of fibres for the most part) is easily understood. As shown in the foregoing pages, this mass of tissue is probably mainly composed of afferent fibres connecting the proboscis with the dorsal cord. As soon as the ventral nerve-cord arose as a concentration of nerve-tissue, this would naturally be followed by another circular concentration in the nervous sheath connecting the ventral cord with the central, invaginated, nervous system, also as an afferent mechanism.

In all probability the enormous increase in size of the larger species was a comparatively recently acquired feature, as also the peculiar odours which they emit; to this latter power it is possibly not too much to attribute the preservation of such a group.

Of the Cephalochorda.—The relations of the Cephalochorda is the next subject for consideration.

The young Balanoglossus agrees with Amphioxus, especially in the following anatomical features :—

(1) The digging mouth.

(2) The repetition and folding of the gill-slits.

(3) The repetition of the generative organs.

(4) The peculiar fate and remarkable asymmetry of the anterior mesoblastic pouch and proboscis pore.

(5) The presence of atrial folds.

(6) The absence of (*a*) any developed sense organs; (*b*) any excretory glands differentiated as such.

(7) In the presence of excretory tubes opening into the atrial cavity.

On the other hand it differs from it in—

(1) The relative size of the præoral lobe.

(2) The degree of its mesoblastic repetition.

(3) The degree of the invagination of its nervous system and the extent of the neural tube.

(4) The extent and degree of isolation of its notochord.

(5) The extent of the atrial folds.

(6) The absence in B. Kowalevskii of any definite liver sacculi, and the presence in B. minutus, &c., of liver saccules differing from those of Amphioxus.

The points of resemblance taken together are so considerable as to suggest that they were possessed by a common ancestor of the Hemichordata and Cephalochorda. On the other hand, the points of difference are nearly all differences of degree, and (1), (2), (3), (4), (6) are points in which the Vertebrata agree with Amphioxus. In the case of (5), however, the Vertebrata more nearly agree with Balanoglossus.

Of the Vertebrata.—The common ancestor, then, of the Cephalochorda and the Vertebrata may be presumed to have possessed the features of mesoblastic repetition, invaginated nerve-cord, and consequent extension of the neural tube, raised, so to speak, to the degree in which they are found in both those divisions. Also it may be believed that the præoral lobe had somewhat diminished and that the atrial folds were still small. The origin of such a liver as that of Amphioxus, as a specialisation of part of the wall of the digestive region of a young B. Kowalevskii is easy to imagine, for the histology of these two tissues is still almost identical. [The presence of peculiar liver saccules in B. minutus, &c., presents no difficulties, as their absence in the more primitive B. Kowalevskii shows that they have arisen within the limits of the group.] Animals possessing those features would answer nearly to the Protochordata of Balfour, though the structures now attributed to it are somewhat different.

The Protochordata thus constituted would then differ from the Enteropneusta in the possession of a serially-repeated mesoblast, in addition to serially-repeated gill-slits, and possibly generative organs; also in the complete separation of the nervous system and notochord. The serial repetition of the gill-slits, the small operculum, &c., they must be presumed to

have acquired from the ancestor common to them and the Enteropneusta.

In this way the connection of the Protovertebrata of Balfour with the other division becomes explicable on the new facts derived from the Enteropneusta.

The peculiar fact that so many of the features of the Enteropneusta differ from those of the Cephalochorda in degree of expression only is very remarkable, and suggest that their further evolution towards the Protochordate type proceeded by correlated variations affecting the several systems.

From the Protovertebrata thus constituted, which in all probability possessed an unsegmented mesoblastic sheath for the notochord and a brain, the Cyclostomata may be easily derived without the necessity of any hypothesis of great degeneration, which cannot be well supported.

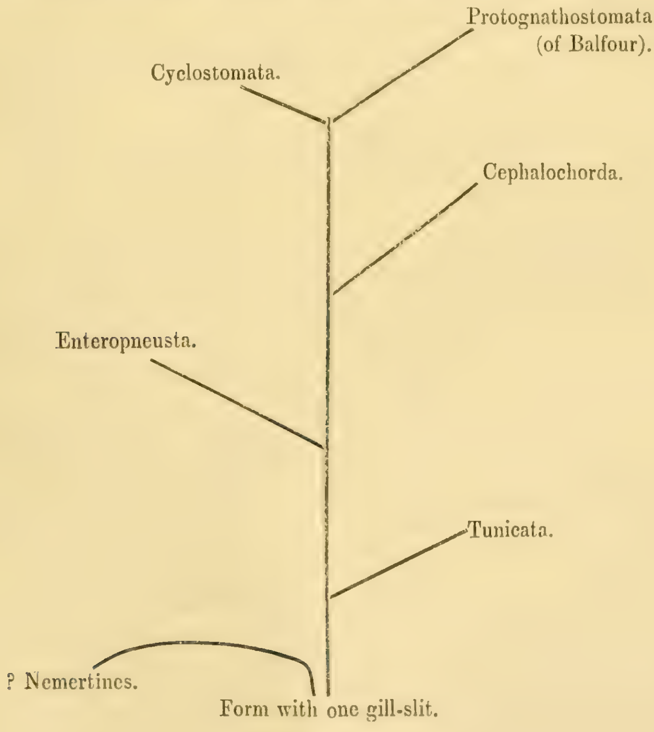
Balfour has fully discussed the question of the origin of his hypothetical group of Protognathostomata, and upon the question of their immediate origin no new light can be thrown.

The above suggestions entail many difficulties. The chief of these is that they involve the hypothesis that the rudiment of the notochord of the Archichordata developed itself as a separate structure, once in the case of the Ascidians, and again in the case of the Protochordata. In the first case, owing to the atrophy of the præoral lobe and use of the tail in swimming, it came to lie in that organ, and in the second case extended through the whole length of the body. Also does this suggestion of the origin of the Tunicates involve the proposition that the rudiment of the dorsal nerve-cord extended itself twice along the body, once in the case of the Ascidians, and again in the case of the Protochordata. If this occurred there is no difficulty in supposing it to have been twice invaginated, this being a more less common feature among nervous systems.

Another difficulty which affects all these suggestions arises from the epiblastic origin of the generative organs of Enteropneusta, in which they resemble the Echinoderms.

Though it is likely that many of the suggestions here made may be shown hereafter to be wrong, still it has seemed well, on the whole, to analyse the facts as they stood, and to endeavour to reconstruct the past stages, whose existence is indicated by the lacunæ in the sequence of these facts, avoiding as far as possible a reliance upon phylogenetic changes of whose occurrence we have no evidence.

The foregoing views are, perhaps, more clearly expressed in the following table, which is not meant so much as a genealogical tree as to serve as an exhibition of the logical relation of the various forms, showing their points of divergence.



Notes on the Development of the Newt (Triton Cristatus).

By

Alice Johnson,
Demonstrator of Biology, Newnham College, Cambridge.

And

Lilian Sheldon,
Bathurst Student, Newnham College, Cambridge.

With Plates XXXIV, XXXV, and XXXVI.

The present paper is a continuation of some observations made by one of us on the early development of the Newt (14). It was then shown that the blastopore of the embryo becomes the permanent anus.

The same discovery has since been made in the Frog by Mr. Spencer (21), in Petromyzon by Mr. Shipley (20), and in Ceratodus by Mr. Caldwell (6). Dr. Gasser stated the same fact with regard to Alytes obstetricans in 1882 (8), in a paper with which the present writers have only recently become acquainted.

The Post-Anal Gut.

The existence of a post-anal gut in the embryos of many Vertebrates appears at first sight an important argument against the view of the identity of the blastopore with the anus, because it would naturally be supposed that the blastopore must be at the extreme hind end of the gut. We find, however, that a post-anal gut is present in the Newt embryo,

and its relations there, as described below, explain this difficulty. In a transverse section taken a very short distance in front of the blastopore (anus), a portion of the dorsal wall of the gut is partially constricted off (fig. 1), and a little further back becomes completely separate (figs. 2 and 3), and may be traced back into the tail as a solid mass of cells, lying just below the notochord. Near the posterior end of the tail this mass dilates (fig. 5), forming a portion which is probably homologous with the caudal vesicle of the post-anal gut in Elasmobranchs (1), and then fuses with the other structures in the tail at the extreme end (figs. 6, 7).

This solid diverticulum of the alimentary canal appears from its relations to be the post-anal gut, and its point of fusion with the notochord and neural canal no doubt represents the neurenteric canal.

At earlier stages the neurenteric canal, which we believe to be always solid in the Newt, though open for a short time in the Frog, is represented by the point at which the fused layers pass into the blastopore. The neurenteric canal is then, roughly speaking, vertical in direction, since the blastopore is situated at the hind end of the ventral surface. When the tail grows out behind the blastopore, the middle point of the vertical neurenteric canal grows out with it, remaining always at its tip, so that the canal becomes, as it were, drawn out into a loop with dorsal and ventral horizontal limbs. The tail is at first composed of undifferentiated tissue, and the differentiation proceeds as usual from before backwards, the dorsal limb of the loop being the medullary canal, and the ventral the post-anal gut. The two limbs are still connected at the posterior end of the tail by the neurenteric canal.

This mode of development seems to us to show that the tail with the post-anal gut is a secondary structure, developed after the permanent anus. The function of the post-anal gut seems to be to provide material for the growth of the tail during embryonic stages before the blood-vessels have formed. With the appearance of the latter, the post-anal gut gradually atrophies, a remnant of it being attached to the rectum just in

front of the anus in a newly hatched larva. At this time it is seen to occupy the normal position of the post-anal gut, being situated between the dorsal aorta and caudal vein.

In the Frog we find a post-anal gut with a wide lumen behind the blastopore. The lumen gradually narrows towards the hind end, and loses itself in the indifferent tissues of the tail. Later the lumen is lost, and the post-anal gut becomes solid.

Dr. Gasser gives an account of a post-anal gut in Alytes (8) like that of the Newt. The lumen of the alimentary canal is continued a very short way into it, and the rest forms a solid cord in the tail. There is no open neurenteric canal in Alytes.

A post-anal gut of the same kind has been described by Mr. Shipley in Petromyzon (20).

The Stomodæum and Pituitary Body.

The stomodæum developes as a solid ingrowth of the inner layer of the epiblast just in front of the anterior wall of the fore-gut (fig. 12). The lower part of the ingrowth fuses with the fore-gut (figs. 14, 8, 9) while the upper projects freely and forms the pituitary body (fig. 14). In fig. 8, which represents an oblique transverse section, the relations of the pituitary body to the stomodæum and fore-gut may be clearly seen. It grows upwards and applies itself closely to the infundibulum, curling round it (fig. 14) and forming an indentation in its floor (figs. 38, 37, 36). The extreme end of the pituitary body is shown in fig. 38, where it is hardly distinguishable from the infundibulum.

The stomodæum fuses with the fore-gut at a very early stage, but no actual perforation is formed until a short time after hatching. The region of fusion takes on gradually the shape of the adult mouth, becoming first elongated transversely, and then horseshoe shaped, with the concavity of the horseshoe directed backwards. The consequence is that, in transverse sections of late stages, the mouth appears to consist

of two lateral parts, which are the limbs of the horse-shoe. We find that the pituitary body and stomodæum develope in exactly the same way in the Frog as in the Newt.

The pituitary body has been described as originating from a solid ingrowth of epiblast in Teleosteans by Hoffmann (13), and it seems to arise somewhat similarly in Lepidosteus (2). Götte also describes the same method of development in Bombinator (9). (See his figs. 127, 128, 250, 252, 292, 293, 298, and 305.)

The Thyroid Body.

From the hind end of the stomodæum proceeds a solid cord of cells continuous along its dorsal border with the fore-gut (figs. 9, 10, 11). This is the thyroid body. Later a groove is continued into it from the fore-gut, and its hind part becomes a tube by the folding over of the edges of the groove. Subsequently the hind end is completely constricted off from the gut. We have not followed its development further. Scott and Osborn (19) described it as being formed from a fusion of hypoblast and epiblast in the median ventral line. We think that this fusion is the stomodæum, with which the thyroid is continuous at its front end, and that the thyroid itself is developed in a perfectly normal manner.

Development of Peripheral Nervous System.

There is no trace of the peripheral nervous system until the neural canal has completely closed and become separate from the external epiblast. Fig. 15 represents a transverse section through an embryo of a stage just before the closure, showing the epiblast in close contact with the neural canal, with which its two layers are of necessity continuous at this time.

The appearance of the peripheral nervous system is preceded by the formation of a neural ridge. In an embryo in which this is first seen, the neural canal has lost all connection with the epiblast in the region of the neural ridge, but remains connected with it in the median dorsal line behind the ridge,

while still further back the closure of the neural canal is not yet complete. The neural ridge now extends through the head (fig. 16) and the anterior part of the trunk (fig. 13).

It may be here stated briefly that, as far as our observations extend, the development of the *spinal nerves* is perfectly normal. The neural ridge is prolonged at regular intervals into nerves, which grow down between the medullary canal and muscle-plates. The upper part of each nerve developes a ganglion, and the ventral root is formed later, whether as an outgrowth from the medullary canal or from the ganglion we are unable to say.

After our discovery of the neural ridge, we found that we had been so far anticipated by Bedot (5), who described in detail the development of the spinal nerves in the Newt. Our observations only confirm his on this point.

The *Cranial nerves*, like the spinal, arise as paired lateral outgrowths of the neural ridge, being completely separate from the epiblast. Figs. 17, 18, and 19 illustrate those outgrowths, which give rise respectively to the 3rd, 5th, and 7th nerves. The 7th and 8th nerves are at first fused, and the common rudiment may be called, for convenience of description, the *Facio-auditory nerve*.

The *Trigeminal* nerve (fig. 18) is an outgrowth from the dorsal surface of the brain, and is directed outwards and downwards towards a lateral thickening of the epiblast, which is cut transversely on one side of the section, and more obliquely, so as to appear longer, on the other side.

The *Facio-auditory* has the same relations to the brain as the Trigeminal, and, like it, is directed outwards and downwards towards a lateral epiblastic thickening. The *9th nerve* grows out similarly towards a corresponding epiblastic thickening. These thickenings are situated slightly above the level of the notochord, and are destined to give rise to the mucous canals of the head. It will be most convenient to take the future history of the nerves separately.

The *3rd nerve* is seen at a later stage in fig. 20. Its point of attachment has been shifted down the side of the

brain, and the nerve is directed forwards towards the eye. We have not ascertained whether or no there is any sensory thickening of the epiblast corresponding to it, but it seems possible that the ciliary ganglion may be fused with the Gasserian, as is stated by Mr. Beard (4) to be the case in the Frog. It would thus not have a separate sense organ of its own.

The Trigeminal nerve grows downwards from the brain till it reaches the level of the sensory epiblastic thickening, and then fuses with it (fig. 21). The point of fusion constitutes the Gasserian ganglion together with the sensory thickening. It is not possible to decide if the epiblast actually takes part in the formation of the ganglion. The mere presence of dividing nuclei in this region, as insisted on by Mr. Beard, seems to us to prove nothing, since all the tissues of the body are actively growing, and consequently contain numbers of such nuclei. We are inclined, therefore, to think that the fusion of the nerve with the epiblast is merely a case of innervation of a sense organ, exactly comparable to what occurs in the nose and ear, and that, in all such cases, the nerve-elements are derived from the brain and the sense elements from the epiblast. Professor Marshall has shown how early this fusion occurs in the case of the ear in the Chick (16).

The root of the 5th nerve is at first attached to the dorsal surface of the brain (fig. 18). Later, the surface of attachment widens out and extends further down the side (fig. 22), and then gradually becomes confined to a small area situated about half way down the brain (fig. 23). The point of attachment is thus shifted downwards, no secondary attachment being formed in this case while the first is lost, as has been described by Professor Marshall in the Chick (16) and in Scyllium (17).

The Gasserian ganglion is for a short time fused into one mass with the sensory epiblast. Soon it begins to sink deeper into the body, but remains attached to the surface by a cord of cells, which constitutes the dorsal branch (ophthalmic) of

the 5th nerve (fig. 24). At the same time a nerve grows down from the ganglion, which soon divides into two branches, a posterior, the inferior maxillary, shown in figs. 24 and 26, and an anterior, the superior maxillary, shown in fig. 24.

The Facio-auditory nerve grows downwards towards its corresponding sensory thickening, and fuses with it at two points, one behind the other. The anterior of these we interpret as the sense organ belonging to the 7th nerve, and the posterior as the ear. There is only a very short distance between them, along which the nerve is not fused. In a later stage, shown in fig. 37, the ear is seen to be clearly distinguishable from the sense organ of the 7th nerve, the ganglion of which is still fused with the skin, while the ear itself is completely separate, forming a simple closed vesicle (fig. 36). The main trunk of the 7th passes on downwards, and fuses with the epiblast of the dorsal wall of the first visceral cleft (figs. 37 and 36). Afterwards, this second connection with the epiblast is lost, and the nerve divides into two branches, one behind and one in front of the first cleft (figs. 26, 31, and 32). At the same time the ganglion on the upper part of the trunk has sunk deeper into the body, remaining attached to the sensory thickening by a cord of cells constituting the dorsal branch (ophthalmic) of the 7th nerve (fig. 31).

The facio-auditory nerve is now attached to the brain by two roots, one behind the other; the anterior is shown in fig. 26, and its connection with the ganglion and præ- and post-branchial branches shows it to be the 7th nerve-root; the posterior passes into the walls of the auditory vesicle (fig. 31), and is therefore the 8th nerve.

The 9th nerve fuses with its corresponding sensory epithelium soon after its origin (fig. 27). The main trunk then passes on and fuses with the epiblast of the 2nd gill-cleft, as shown at a later stage in fig. 28. The root by this time has shifted downwards from the dorsal surface of the brain. The subsequent course of events is exactly the same as in the case of the 7th nerve. The ganglion retreats further from the surface of the body, remaining attached by the dorsal nerve to

the sense organ (fig. 34), and the ventral portion of the main trunk divides into two branches, the post-branchial (fig. 35) behind the second gill-cleft, and the præ-branchial (fig. 33) in front of it.

The Vagus arises from the brain in the same manner as the other cranial nerves, but we have not traced its further development.

Development of Nerves in the Frog.

We have made a few observations on the development of the nerves in the Frog in some series of sections cut by Mr. Durham, and very kindly lent to us. Our observations, as far as they extend, confirm in every respect what we have described in the Newt. A neural ridge is formed on the dorsal surface of the medullary canal after it has separated from the epiblast, as shown in fig. 30, representing part of a transverse section through the hind region of the trunk of an embryo. In this embryo the neural ridge extended through the trunk, but was less distinct in the head, where the nerves had begun to form as outgrowths from it. Fig. 29 shows the origin of the facio-auditory nerve. Its small size shows that it must be at a very early stage. It is growing on each side from the dorsal surface of the brain towards the auditory vesicle, which is beginning to develope from the inner layer of the epiblast. It seems to us that the whole appearance is inconsistent with the view that the nerve has split off from the epiblast, as Mr. Spencer asserts (21).

Historical and Critical.

Our observations are, on the whole, consistent with the account of the derivation of nerves first put forward by Professor Balfour in 1876 (1), afterwards confirmed by Professor Marshall in other types, and since generally accepted. They do not lend any support to the peculiar view of His, as to the presence of a "Zwischenstrang" (11).

Sagemehl (18) derives the spinal nerves in the Frog from a

neural ridge, but states that they become detached later from the spinal canal, and subsequently joined to it by the dorsal and ventral roots. Bedot (5) states that in the Newt the connection is never broken, and our researches lead us to agree with him on this point.

Hoffmann (12) describes the spinal nerves in Teleosteans as growing from a neural ridge, but appears to think that the cranial nerves, which arise before the neural canal is closed, are, partially at least, derived from the adjacent epiblast.

O. Hertwig (10), in a few scattered observations on the spinal nerves of the Frog, is inclined to support His' view. More recently the theory of the derivation of the whole or greater part of the cranial nerves from the epiblast has been supported by Mr. Spencer (21) and Mr. Beard (4). This view is a revival of that held by Götte (9). Mr. Spencer asserts that the whole nerve, including root and ganglion, is, in the Frog, split off from the nervous layer of the epiblast. If this be so, all the branches must ultimately be derived from the same source. Mr. Beard confirms him in this statement, and figures one section showing a thickened mass of epiblast continuous dorsally with the still open neural canal, but there is nothing to show that this thickening becomes a nerve. Such a split, as is figured between it and the external layer of epiblast, very often occurs in imperfectly preserved specimens. We find no such thickenings in Newt embryos of similar stages, a typical section of which is shown in fig. 15, and our observations on the Frog lead us to doubt the accuracy of Mr. Spencer's account. We have attempted to show that it is, at all events, not universally true for Amphibia, as Mr. Beard assumes.

Mr. Beard has described in Elasmobranchs (4) a fusion of the typical cranial nerve with the sense organ of its segment. This corresponds with the dorsal fusions found by us in the Newt. The ventral fusion of the nerve with the gill-cleft, as described above in the Newt, corresponds to the second fusion found by van Wijhe in Elasmobranchs (22), and to the ventral fusion found by Froriep in Mammals (7). Mr. Beard considers

that, in Elasmobranchs, all the main branches of the nerve except the post-branchial and the part between the ganglion and the brain are split off from the epiblast. Van Wijhe holds that the epiblast takes some share in the formation of the ganglion at least, while Froriep expresses doubt as to this point, comparing the fusion to the similar fusion of nerve-cells and epithelium cells in the ear. We are strongly inclined to the last view. Professor Marshall (16) has shown how very early the nerve-cells of the ear become indistinguishably fused with it, and there seems no reason why this should not be the case with other sense organs. As to the splitting off of the nerve-trunks from the skin, Mr. Beard's observations and deductions seem to us inconclusive.

In Elasmobranchs Professor Balfour mentioned and figured a fusion between the mucous canals of the head and the nerves supplying them, no line of demarcation existing between the two structures (v. loc. cit., pp. 144, 145, plate xii, fig. 7). He describes this as occurring first in his Stage P, but it is possible that it may take place rather earlier in the Elasmobranchs, as it certainly does in the Newt. Mr. Beard seems to have detected the earlier fusion in Elasmobranchs, and to be unaware that the fact of the fusion was described by Professor Balfour, who found that the nerves were all derived from the brain outgrowths, as we believe to be the case in the Newt. It appears to us that the epiblast in this animal takes no part in the formation of the ganglion or nerve branches, and that the special nerve to the sense organ is an outgrowth from the ganglion, advancing pari passu with the withdrawal of the latter from the surface, so that there is at no time any break in the connection between the sense organ and its nerve supply. The withdrawal of the ganglion and formation of the nerve is only a result of the differentiation of the nerve supply into a ganglionic and a fibrous part.

The disposition of these sense organs seems to us a very insecure guide to the segmentation of the head. Mr. Beard considers that the relations of the sense organs to the gill-clefts shows them to be of segmental value, since they are in

some cases situated one above each gill-cleft. At the same time he is obliged to assume the existence of more than one now aborted gill-cleft, in order to account for the number of the sense-organs. If the proof of the segmental value of the sense organs is to depend on the number of the gill-clefts, and the number of the gill-clefts is in turn to depend on the segmental value of the sense organs, it is difficult to discern which is the basis of the argument. Malbranc (15) shows that even in the embryo multiplication of the sense organs by division may occur, so that the number of them seems to be indefinitely variable; and Mr. Beard himself has described such a division in the case of the sense organs of the facial nerve. It seems, therefore, that there was primitively only one such sense organ in this case, and that one cannot depend on the number of the sense organs at any but the very earliest stages, if even then, as indicating segmentation.

Summary of Observations.

1. A solid post-anal gut is formed behind the blastopore (anus), growing out into the tail, and fusing with the undifferentiated tissues at its posterior end. The fusion of hypoblast and epiblast in this region represents the neurenteric canal.

2. In the Frog the post-anal gut is at first hollow, but afterwards becomes solid.

3. The stomodæum and pituitary body are derived from a solid ingrowth of the inner layer of the epiblast. The hind part of this ingrowth fuses with the front wall of the fore-gut, but the perforation to form the actual mouth does not appear till after hatching. The pituitary body grows upwards as a solid cord, and applies itself to the infundibulum in the ordinary manner.

4. From the hind border of the stomodæum proceeds a solid rod of cells, which constitutes the thyroid body, and is developed from the cells of the middle ventral line of the fore-gut.

5. The development of the peripheral nervous system is

preceded by the appearance of a neural ridge, extending along the whole length of the body.

6. The spinal nerves grow out from the neural ridge, and pass downwards between the neural canal and muscle plates.

7. The cranial nerves also grow out from the neural ridge, but are nearer to the surface than the spinal nerves, owing to the absence of muscle plates in the head.

8. When each has attained a certain length it fuses with a thickening of the epiblast, situated some distance above the level of the notochord. (This is the case with the 5th, 7th, and 9th nerves, and probably also with the vagus.)

9. At the point of fusion there is a thickening of the nerve-trunk, forming a ganglion, which afterwards recedes from the surface, remaining, however, attached to the sense organ by a nerve.

10. The main trunk of the nerve passes on, and, in the cases of the 7th and 9th nerves, fuses again with the epiblast of the dorsal wall of the corresponding gill-cleft. Later, the nerve becomes detached from the epiblast, and gives off two branches, one behind and one in front of the gill-cleft.

11. The 5th nerve has no such second (ventral) fusion with the epiblast, but divides below its first (dorsal) fusion into two branches, the superior and inferior maxillary.

12. In the Frog a neural ridge is present at an early stage, just after the closure of the neural canal. The facio-auditory nerve grows out of the brain, and it is therefore probable that the other cranial nerves have the same origin.

N.B.—Our figures are diagrammatic in so far that the outlines of the cells were not perfectly apparent in all sections. This appeared to us to be due to bad preservation, as the better the specimens were preserved the more distinct and complete were the cell outlines. It was generally possible to draw them accurately with a camera and Zeiss obj. D, oc. 2. We have therefore represented them throughout as distinct.

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EXPLANATION OF PLATES XXXIV, XXXV, and XXXVI,

Illustrating Alice Johnson's and Lilian Sheldon's Paper "On the Development of the Newt (Triton cristatus)."

All the figures represent single sections. They were drawn with a Zeiss's camera lucida and Zeiss's obj. A, oc. 2, except Figs. 1, 2, and 3, which were drawn with obj. B, oc. 2; Figs. 6 and 7 with obj. C, oc. 2; and Figs. 4, 5 and 30 with obj. C C, oc. 2. Fig. 12 was drawn with obj. A, oc. 2, and afterwards reduced by one half; and Figs. 13, 16, 17, 18, 36, 37 and 38 were drawn with obj. A, oc. 2, and afterwards reduced by one third.

N.B.—Grey denotes epiblast, and organs derived from it; brown denotes mesoblast; and yellow denotes hypoblast, and organs derived from it.

Alphabetical List of Reference Letters.

Aud. Ear. *Bl.* Blastopore. *Ch.* Notochord. *F. Aud. rt.* Root of Facio-auditory nerve. *F. B.* Fore-brain. *F. G.* Fore-gut. *Gass.* Gasserian ganglion. *G. VII.* Ganglion of 7th nerve. *G. IX.* Ganglion of 9th nerve. *H. B.* Hind-brain. *H. G.* Hind-gut. *Inf.* Infundibulum. *Lat. V.* Thickening of nervous layer of epiblast to form sense organ corresponding to 5th nerve. *Lat. VII.* Thickening of nervous layer of epiblast to form sense organ corresponding to 7th nerve. *Lat. IX.* Thickening of nervous layer of epiblast to form sense organ corresponding to 9th nerve. *M. B.* Mid-brain. *Mes.* Mesoblast. *N. C.* Neural ridge. *Olf.* Olfactory epithelium. *O. V.* Optic vesicle. *P. a. g.* Post-anal gut. *Pit.* Pituitary body. *Sp. c.* Spinal cord. *St.* Stomodæum. *Thal.* Thalamencephalon. *Thy.* Thyroid body. *V. C. I.* First visceral cleft. *V. C. II.* Second visceral cleft. *III.* Third nerve. *V.* Fifth nerve. *VII.* Seventh nerve. *IX.* Ninth nerve. *III.rt.* Root of 3rd nerve. *V rt.* Root of 5th nerve. *VII rt.* Root of 7th nerve. *VIII rt.* Root of 8th nerve. *IX rt.* Root of 9th nerve. *V d.* Dorsal branch of 5th nerve. *VII d.* Dorsal branch of 7th nerve. *IX d.* Dorsal branch of 9th nerve. *V sup. max.* Superior maxillary branch of 5th nerve. *V inf. max.* Inferior

maxillary branch of 5th nerve. *VII post-br.* Post-branchial branch of 7th nerve. *IX post-br.* Post-branchial branch of 9th nerve. *VII præ-br.* Præ-branchial branch of 7th nerve. *IX præ-br.* Præ-branchial branch of 9th nerve. *x.* Fusion of 7th nerve with epiblast of gill-cleft.

Figs. 1—7.—Series of transverse sections through an embryo, to show the relations of the post-anal gut to the hind-gut; Fig. 1 being the most anterior, and Fig. 7 the most posterior.

Fig. 1. A little in front of the blastopore, to show the origin of the post-anal gut from the hind-gut.

Fig. 2. Showing the post-anal gut completely separated from the hind-gut.

Fig. 3. Through the blastopore.

Fig. 4. Behind the blastopore.

Fig. 5. Showing dilatation of the solid post-anal gut near the hind end of the tail.

Fig. 6. Showing fusion of the post-anal gut with the notochord and the neural canal.

Fig. 7. Showing fusion of the mesoblast with the other layers near the extreme end of the tail.

Figs. 8—11 are taken from one series of transverse sections through the anterior end of an embryo, to show the origin of the stomodæum, the pituitary body, and thyroid body. Fig. 8 being the most anterior, and Fig. 11 the most posterior.

Fig. 8. Showing the origin of the stomodæum and pituitary body, and the fusion of the former with the anterior wall of the fore-gut. It also shows the root of the fascio-auditory nerve, and its ventral fusion with the epiblast.

Fig. 9. Showing the hind end of the stomodæum.

Fig. 10. Showing the anterior end of the thyroid body as a solid rod of cells attached to the ventral wall of the fore-gut.

Fig. 11. Showing the thyroid body near its posterior end.

Fig. 12.—Longitudinal vertical section through the head end of an embryo, to show the origin of the stomodæum and pituitary body as a solid ingrowth of epiblast in front of the fore-gut.

Fig. 13.—Transverse section through the trunk of an embryo shortly after the closure of the medullary canal, to show the neural ridge.

Fig 14.—Longitudinal vertical section through the head end of a somewhat older embryo than that from which Fig. 12 was taken, to show the relations of the stomodæum and the pituitary body to the fore-gut, infundibulum, and notochord.

Fig. 15.—Transverse section through the trunk of an embryo shortly before the closure of the medullary canal, showing the epiblast continuous dorsally with it.

Fig. 16.—Transverse section through the head end of an embryo at a stage shortly after the closure of the medullary canal, to show the neural ridge in the brain. Owing to the cranial flexure, all three divisions of the brain are cut through.

Fig. 17.—Transverse section through an embryo slightly older than that from which Fig. 16 was taken, showing the origin of the 3rd nerve as a paired outgrowth from the neural ridge in the mid-brain.

Fig. 18.—Transverse section through the same embryo as that from which Fig. 17 was taken, showing the origin of the 5th nerve from the neural ridge in the hind-brain. The lateral thickening of epiblast on each side is shown.

Fig. 19.—Transverse section through the hind-brain, to show the origin of the 7th nerve as a paired lateral outgrowth of the neural ridge. The lateral thickening of epiblast, which will give rise to the ear and sense-organ of the 7th nerve, is shown on each side.

Fig. 20.—Transverse section through a somewhat older embryo, showing that the root of the 3rd nerve has shifted to the sides of the mid-brain.

Fig. 21.—Transverse section, showing the attachment of the Gasserian ganglion to the epiblastic thickening forming the sense-organ corresponding to the 5th nerve.

Fig. 22.—Slightly oblique transverse section, to show the shifting of the root of the 5th nerve; its attachment is seen to extend continuously from the summit of the brain to a point some way down its side.

Fig. 23.—Transverse section through an older embryo, to show the shifting of the root of the 5th nerve. The nerve is now connected only with a small area of the side-wall of the brain.

Fig. 24.—Transverse section through a still older embryo, showing on the right side the superior maxillary and dorsal branches of the 5th nerve growing out from the Gasserian ganglion. On the left the Gasserian ganglion and inferior maxillary are shown.

Fig. 25.—Transverse section through a young embryo, showing on the left the root of the facio-auditory nerve and its fusion with the epiblast; on the right the auditory epithelium and ventral continuation of the nerve.

Fig. 26.—Transverse section through the same embryo as that from which Fig. 24 was taken, but slightly posterior to it. It shows on the right the Gasserian ganglion and inferior maxillary branch of the 5th nerve; on the left the root, ganglion, and præ-branchial branch of the 7th nerve.

Fig. 27.—Transverse section through a young embryo, showing the root of the 9th nerve and its fusion with the lateral thickening of epiblast corresponding to it. On the right the nerve is seen passing on to the 2nd visceral cleft.

Fig. 28.—Transverse section through a somewhat older embryo. It shows on the right the root, ganglion, and main branch of the 9th nerve, the last fusing with the epiblast of the dorsal wall of the 2nd visceral cleft. On the left only the main branch and its fusion are seen.

Fig. 29.—Transverse section through the head end of a Frog embryo, showing the origin of the facio-auditory nerve as an outgrowth from the dorsal surface of the hind-brain. The thickening of the nervous layer of epiblast to form the ear is also shown.

Fig. 30.—Transverse section through the posterior part of the trunk of the same Frog embryo shortly after the closure of the medullary canal, to show the neural ridge.

Figs. 31—35.—Transverse sections through the same embryo as that from which Figs. 24 and 26 were taken, but posterior to them.

Fig. 31. Showing on the right the ganglion and the dorsal and præ-branchial branches of the 7th nerve; on the left the ear and the root of the 8th nerve, and the 1st visceral cleft.

Fig. 32. Showing on the right the ganglion of the 7th nerve; on the left the ear and the post-branchial branch of the 7th nerve.

Fig. 33. Showing on the right the ganglion and præ-branchial branch of the 7th nerve; on the left the ganglion and præ-branchial branch of the 9th nerve.

Fig. 34. Showing on the right the ganglion and præ-branchial branch of the 7th nerve; on the left the root, ganglion, and dorsal branch of the 9th nerve, and also the 2nd visceral cleft.

Fig. 35. Showing on the right the ear and post-branchial branch of the 7th nerve; on the left the ganglion and post-branchial branch of the 9th nerve.

Figs. 36—38.—Transverse sections through the head end of an embryo, to show the relation of the pituitary body to the fore-gut and infundibulum.

Fig. 36. Showing the fusion of the posterior face of the pituitary body with the wall of the fore-gut. It also shows the ear and the ventral fusion of the 7th nerve with the epiblast of the dorsal wall of the 1st visceral cleft.

Fig. 37. Slightly anterior to the preceding, showing the pituitary body in close contact with the wall of the infundibulum. It also shows on the left side the ear, the ganglion of the 7th nerve, and the ventral fusion of the nerve with the epiblast.

Fig. 38. Showing the free tip of the pituitary body in close contact with the wall of the infundibulum.

Recent Researches on Oogenesis.

By

Arthur Thomson.

I. Polar Globules and Extrusions from Reproductive Cells.

In Balfour's convenient summary, which may be regarded as representing the state of scientific knowledge in 1880, polar globules are defined as one or two cells which appear on the surface of the ovum before or after fertilisation, and which arise by a regular process of division from the germinal vesicle. As to their physiological import it is further suggested that in their extrusion certain important constituents are removed from the germinal vesicle, without which it is incomplete and incapable of further development, unless these are again supplied by the spermatic nucleus, but retaining which the ovum is capable of developing parthenogenetically. The somewhat too teleologically-expressed observation "that the function of forming polar cells has been acquired by the ovum for the express purpose of preventing parthenogenesis," is supported by reference to the fact that parthenogenesis is specially characteristic of Arthropoda and Rotifera, "the only two groups in which polar bodies have not so far been satisfactorily observed."

In attempting to summarise the most important recent researches on the history and nature of polar globules, it will be convenient to report these as they concern (1) the occurrence and history of polar cells, and of other extrusions from the reproductive cell; (2) the morphological import of these bodies; and (3) their physiological rationale.

The Occurrence of Polar Cells.—Little has been done since Balfour wrote, in the way of extending our knowledge of the actual occurrence of polar cells in the different groups. Grobben has, however, observed what seems to be an undeniable polar cell in Cetochilus, Billet has reported their occurrence in Rotifera, and Weismann (1) has lately announced the existence of a distinct polar cell in the summer ova of some Daphniids.

Other Extruded Elements.—On the other hand, numerous observers have noted the extrusion of protoplasmic elements from the ovum, which resemble in some respects the true polar cells, with which they have been repeatedly compared and confused. In the first rank among these, and as yet unique, are the pole cells of insects. They were first described by Robin in 1862, who compared them to true polar cells (vesicules directrices, Richtungskörperchen, directive bodies), and stated that they were incorporated in the blastoderm and utilised. Through the subsequent researches of Leuckart, Metschnikoff, Grimm, Weismann, and Balbiani, it has been shown that (following Balbiani's (2) last report, 1885) two distinctly cellular elements, with nuclei apparently derived from the germinal vesicle, appear, usually successively, at the posterior pole of the ovum. In their further history they differ widely from polar cells, for they immediately divide into eight, and, after the blastoderm is differentiated, are carried in again by an invagination, and become, after fusing into four, the male or female reproductive organs. The re-entrance is denied by some but seems conclusively established. Weismann has also described distinct "Richtungskörperchen," but this is not corroborated by Balbiani, who notes the likelihood of mistake caused by the presence of "protoplasmic drops" at both poles, which originate according to him from the contraction of the vitellus. Weismann has, however, noted the presence of mobile amœboid bodies in addition to the pole cells and alleged directive bodies.

Non-cellular extrusions.—Apart from the special case of insects, extrusions somewhat comparable with polar cells,

but usually distinctly non-cellular, have been observed in the ova of many animals. Such cases are discussed at length in a recent memoir by Sabatier (3) (1884). His own observations relate especially to the ova of Buccinum and Lymnæus, in which besides an extrusion of protoplasm associated with the formation and division of a nuclear spindle (polar cell formation), other protrusions of an apparently subordinate nature may occur. Globules are extruded at various positions on the surface of the ovum, for instance at the pole opposite to that at which the polar cells appear, and these expressed globules may multiply by division outside the ovum. Sabatier maintains the occurrence of what he calls centrifugal movements of portions of the protoplasm in the ova of both Vertebrates and Invertebrates, e.g. Holothuria, Helix pagurus, Geophilus, Rana, &c., and interprets numerous observations in terms of his theory, e. g. such phenomena as follicular cell formation, in which, as we shall see, he maintains an intra-vitelline but non-nuclear mode of origin. He distinguishes three kinds of globules.

(1) Globules précoces ou du début.—Initiatory extrusions, which usually form the elements of the follicle.

(2) Globules tardifs.—Late extrusions shortly before the maturation of the ovum.

(3) Globules de maturation parfaite.—Differing from the two preceding in being associated with karyokinetic changes in the nucleus, which remains passive and central in the two preceding cases. These are the true polar cells.

(3 *a*) In some cases elements are extruded at maturation without nuclear participation.

Sabatier traces the history of these different extrusions.

(1) The initiative bodies or "globules précoces" in the ova of Ascidians, Vertebrates, some Molluscs, some Annelides, Gephyreans, Arthropods, &c., form outside the egg either irregularly distributed masses of granular protoplasm or a complete envelope. In other cases they disappear very soon, disorganised and reabsorbed, between the ovum and the surrounding capsule.

(2) The elements extruded at a later stage, the "globules tardifs," result in the granular cells of Ascidians, probably too in the globules of the shells of winter eggs of Cladocera (Weismann), in the curious envelope of the ova of Chiton (Hering), in the peripheral hyaline bodies on the eggs of Phanerocarpous medusæ (Giard). Giard has observed ('Comptes rendus,' March, 1877) the extrusion of hyaline globules all round the periphery of Rhizostomum, &c.

(3 *a*) He notes also some difficult cases of globules expressed at maturity but without karyokinetic change of the nucleus, e.g. the "voile" in the ova of certain Amphibians, first observed by Max Schultze (1863) and termed fovea germinativa, and since investigated by Bambeke and O. Hertwig. With these it is interesting to compare the yolk globules recently ('Archiv f. mikr. Anat.,' xxvi, December, 1885) demonstrated by Solger on the intracapsular space of certain fish ova.

Mode of Formation.—The classic researches of Fol and Hertwig as to formation of polar cells have been generally confirmed. Van Beneden, however, maintains that in Ascaris megalocephala the division takes place parallel to the long axis of the nuclear spindle, so that two dissimilar halves might result. His results have been ably summarised in a recent number of this Journal. Sabatier agrees with Van Beneden as to the longitudinal division, and maintaining that the extrusion is not directly comparable with ordinary indirect cell-division, asserts the concurrence of two distinct processes, not necessarily related, (1) the formation of a nuclear spindle and consequent division, and (2) the independent expulsion of a more or less considerable mass of circumnuclear protoplasm. As to the further history of the polar cells, it has been observed by Trinchese and others that they sometimes divide each into two and then into four cells. Flemming (' Biol. Centlblt.,' iii, 21, 84) has pointed out the constancy with which, in some cases at least, the polar cells appear at a definite position on the ovum, e. g. in Anodonta the polar cells appear constantly antipodal to the " haft-pole," at which the ovum is attached to the membrane.

Analogous Processes in Plants.—In his last memoir (1884) Strasburger (4) notes numerous instances where portions of the reproductive cell are excluded by extrusion or otherwise from the differentiated result. Thus, in the female cells of Vaucheria and Œdogonium there is an expulsion of the colourless protoplasm which collects at the anterior pole. From the ova of Archegoniatæ, shortly before maturation, a "Bauchkanalzelle" is separated off by ordinary division, and a similar cell is formed in Coniferæ, and irresistibly reminds one, he says, of the polar cell of the animal ovum. In the differentiation of the male cell analogous processes may occur, unused material is extruded along with the spermatozoids from the antheridia of Fucus; a similar remnant is observable in Vaucheria, while the spermatozoids of Archegoniatæ are formed from the nuclear substance round a central vesicle, which retains the unused portions of the spermatocytes. In Salvinia a portion of the protoplasm is at an early stage excluded from the formation of the four spermatocytes. The "secreted body" ("secret-Körper") which Strasburger found in the mother-pollen cell before division, has been collated by different authors (e. g. Nussbaum) with the excentric problematic body (Nebenkörper), which has been described in the spermatogonia of Astacus, Helix, &c., and which seems to disappear as division sets in. Strasburger's recent researches on the ontogeny of pollen grains suggest also similar comparisons with processes in the differentiation of animal sex-cells. In Gymnosperms the prime pollen-cell or "progame" divides into a smaller vegetative and a larger reproductive portion; the latter may again divide once or twice, and the result is a generative cell, with, perhaps, three rapidly dwindling vegetative cells. Since these vegetative cells are successively divided off from the reproductive they are for this and other reasons not comparable with a prothallium. In Angiosperms the process is essentially similar. In all cases the vegetative nuclei, separated off from the reproductive, disappear without playing any rôle and without dividing.

In regard to the extrusions formerly cited, it must be allowed

that Strasburger insists on their arbitrary occurrence, distinctly present in one form, but apparently altogether absent in others nearly allied ; yet it is not necessary to go so far as he seems to do in doubting whether such elements as Bauchkanalzelle and polar cells have really any definite morphological import, since it is easy to understand how a process of slight morphological import, whose persistence is of course conditioned by an immediate physiological necessity, might disappear in any case where the physiological necessity was otherwise satisfied.

Comparison with similar Processes in Spermatogenesis.—The prevalence of such physiological theories as those of Balfour and Minot, according to which the formation of polar cells is an extrusion of male elements from the ovum, has led to a frequent comparison between the latter process and the separation of elements during spermatogenesis. That such comparisons are not necessarily merely physiological, is evident from the homology between ovum and mother-sperm cells[1] (S^1), first emphasised by Reichert in 1847, and since then more or less consistently recognised by various investigators. The frequent close resemblance between the structure and origin of the glands and between the early stages of the sex-cells has been often noted; and, further, Fol, for instance, has maintained that the follicular cells are genetically the strict homologues of spermatoblasts; while Nussbaum ('Archiv f. mikr. Anat.,' xviii), following von la Vallette St. George, collates spermatogonium with ovum, and the follicular envelope (Follikelhaut) of the spermatogonium with the follicular epithelium of the ovum, maintaining that in Amphibia and Teleostei spermatogonium and follicular cells arise from the morula-like division of the nucleus of a primitive

[1] In the confused state of the nomenclature of spermatogenesis, it is convenient to denote the undifferentiated sperms, spermatocytes, &c., as (S^0), the cells from the division of which these result, spermatogonia, spermatoblasts, &c., as (S^1) the mother-cells of these (S^2). See Geddes and Arthur Thomson on 'History and Theory of Spermatogenesis,' Proc. Roy. Soc. Edin., 1886.

cell, just as has been maintained in regard to the ovum. It has been lately attempted to carry the comparison further by collating different forms of spermatogenesis with different modes of ovum segmentation.

If any process in spermatogenesis can be morphologically as well as physiologically compared with polar cell-formation, these must occur in the mother-sperm cell or spermatogonium, while other phenomena observed in the spermatocytes or undifferentiated sperms may admit of physiological comparison. In their account of the spermatogenesis of Ascaris megalocephala, van Beneden and Julin (5) describe in the region where the spermatogonia are formed at the expense of their mother-cells or spermatomeres, certain corpuscles which they compare to polar cells. Two of these "residual globules" are, according to them, expelled by the spermatomeres during their nuclear metamorphosis preceding division, and they believe that the expulsion is made in a manner similar to that in which they maintain that polar cells are formed, so that there is an actual extrusion of half the elements of the nuclear plate. This process, and their account of polar cell-formation, is not, however, corroborated, and is vigorously contested, e. g. by Strasburger. In the spermatogonia themselves, or in what he calls the spermatoblasts in Astacus, Eriphia, and many other Crustacea, Grobben ('Arb.,' Wien, 1878) has described a definite body (Nebenkörper) occurring in the protoplasm, sometimes far from, sometimes near, or even apparently connected by fine filaments with the nucleus, in regard to which he suggests that it is, perhaps, a portion of the nucleus of the spermatoblast, extruded at maturation, before division into spermatocytes. Semper has also described a similar problematic body in the corneal mother-cells within which his spermatoblasts are formed. A comparison has also been frequently drawn between the polar cells and the portions of spermatoblast which seem to be excluded from a share in the actual formation of spermatocytes, e. g. the "Deckzelle" of Semper, which lies at the base of each mother-cell between the spermatoblasts and the ampulla wall, in regard to whose origin Semper is undecided.

Similarly Minot has drawn a parallel between the basal, according to him, female portion of a spermatoblast and the ovum minus its (male) polar cells, which would thus be physiologically analogous to sperms. On the other hand, according to Weismann, the parallel would be between the surplus "ovogenetic" polar vesicles and the surplus spermatogenetic basal protoplasm and nucleus, between the preponderatingly germinal nucleus of the ovum and the combined nuclei of the spermatocytes. It is, however, impossible to decide as to these parallels until, on the one hand, some method be discovered for demonstrating the physiological similarities of protoplasmic masses, and, on the other, the exact behaviour of the nuclei in spermatogenesis be more satisfactorily known. The cell or cytophore in the centre of a mass of spermatocytes has also been regarded as a separated-off portion of the spermatogonium, and physiologically compared with polar cells, but Voigt ('Arb.,' Würzburg, viii, 85) has recently emphatically denied its cellular nature, and ascribed its origin to the stalk of the spermatogonium cell, which, as the latter divides, comes to lie in the midst of the spermatocytes. In spermatocytes a peculiar body, first described by La Valette St. George (Nebenkern), has been lately the subject of much discussion, some deriving it from the protoplasm and others from the nucleus, some regarding it as separated from the formation of the spermatozoon, and others regarding it as the origin of the sperm cap, or middle portion or even head.

Morphological Import of Polar Cells.—The persistence of such a process as polar cell-formation seems to point to a morphological import, and it is on this aspect of the phenomenon that most recent investigators have concentrated their attention.

The definite cellular nature of the extruded bodies, which is generally acknowledged, the definiteness of their mode of formation, their occasional subsequent division, and the prevalence of their occurrence, point, however, to a distinct morphological import, and that, as Weismann emphasises, of ancient phylogenetic origin, which has of course again to be explained in

terms of physiology. Their physiological import, if discovered, might shed light on the meaning of alleged extrusions among Protozoa and lower plants, while it is in a study of these that the morphological import of polar cells is perhaps most hopefully to be sought. The general theory suggested by Fol, Giard, Mark, Whitman, Flemming, and others, is that polar vesicle formation represents phylogenetically the survival of an asexual or parthenogenetic division, diminishing, according to Mark, for the good of the ovum. Minot suggestively compared the process to the nuclear extrusion alleged to exist in Infusoria.

Bütschli (6) has lately (1884) made a more detailed attempt to determine the morphological import of these cells. Referring to the colonies of sexual cells formed by multiplication in such organisms as Eudorina and Pandorina and Volvox, he suggests that polar cell-formation is an all but obliterated survival of the early colony formation.

Physiological Import.—Speculations as to the physiological meaning of the polar cells are abundant enough. Some regard them as effecting a desirable lessening of the nuclear mass, either to prevent parthenogenesis, or to decrease the disproportion between female and male nucleus. Minot (7) regards them as definitely male elements, retained in parthenogenesis, necessarily excluded to secure sexual differentiation, and compares them with the sperm blastophor, while it has been also maintained that in their extrusion the more passive portion of the germinal vesicle is expelled. Brass ('Zool. Anz.,' 1882) compares them to vacuole contents of amœbæ, and regards them as the excretory products of an actively functioning cell. Since such speculations are for the most part still too indefinite and in some cases too teleological, it will be sufficient to note three of the most recent, those of Sabatier (1884), Strasburger (1884), and Weismann (1885).

As the result of his comparative study of spermato- and oo-genesis, Sabatier (3) was led to observe (1) that the male elements resulted from differentiation in the peripheral or protoplasmic portion of the reproductive cell, while the nuclear

or central portion, forming the core round which the sperms were developed, atrophied and disappeared; (2) that the ovum, on the other hand, resulted from the increasing preponderance of the central nuclear portion, though partly at the expense of the peripheral. From this he was induced to formulate a general theory of sexual polarities, according to which there is in every cellular element an antagonism or different polarity between the central nucleus, and immediately enveloping protoplasm on the one hand and the peripheral protoplasm on the other. These polarities are sexual in nature, the central polarity corresponding to the female element, and the peripheral polarity to the male. Every cell in which the two polarities are maintained in equilibrium is neuter. The predominance of either polarity conditioned by nutrition, &c., makes the cell distinctly unisexual, and the differentiation of sexuality is, according to Sabatier, effected by the, possibly repeated, extrusion of one of the two substances both originally present. The female polarity is centripetal and its tendency is to effect cohesion and integration; the male polarity is always centrifugal and its tendency is to effect separation and dissolution. The normal cell is thus bipolar and ordinary division is inaugurated by a sort of intracellular fertilisation between protoplasm and nucleus, the former taking the initiative. In the reproductive cell the centripetal female polarity is localised in nucleus and central protoplasm (germinal vesicle and ovum proper in female, blastophore sperm in male); the male centrifugal is localised in that portion of the protoplasm at the expense of which (according to Sabatier) the centrifugal elements (follicular cells, polar cells, perivitelline layers, spermatoblasts, &c.) are formed. The extrusion of globules from the ovum is thus the elimination of male substance. In some cases the process is several times repeated, in parthenogenetic ova there is reason to believe that it is less. The early "globules précoces" are in a sense determinative, their expulsion makes the ovum definitely female, though not usually to the necessary extent. Under the influence of persisting male substance the nucleus seg-

ments to form the first true polar cell, at the same time the stimulated protoplasm contracts to eject some of the male element. If in the first division of the nucleus the male element be sufficiently got rid of, the process ends and the nucleus sinks back, if not a second polar cell is formed. The polar cell being mostly male disintegrates, though the presence of some lecithin or nutritive material may enable it to divide into isolated pieces; the ovum does not divide, for it has got rid of all the male substance which alone could render a parthenogenetic division possible.

In his suggestive observation on polar cells, Bauchkanalzellen, and extrusions of other kinds from reproductive cells, Strasburger (4) refers these phenomena to the necessity of securing for the differentiating reproductive nucleus a definite cytoplasmic medium. The expulsion of the polar cell he is inclined to regard as a separation of part of the nutritive plasma, insisting that there is here no separation of male elements, since the results of indirect nuclear division are always two exactly similar twin daughter nuclei. He maintains that the differentiation of ovum or sperm nuclei is not effected by the extrusion of specific elements, but on certain modifications of the nuclear substance, and on certain nutritive conditions between cytoplasma and nuclei, which may be in some cases achieved by an elimination of portions of either.

The last-developed theory of polar cells is due to Weismann (1), who brings the phenomena into relation with his theory of the continuity of the germ plasma. Starting from the conception that the ovum is a histologically differentiated, nutritive-glandular cell, which must have a specific nuclear, histogenetic, or ovogenetic plasma besides the germ plasma, he maintains that the former predominates in the young ovum, while the latter is present only in small though increasing quantity. That the germ plasma may predominate and the development begin, some of the ovogenetic plasma must be removed from the ovum, and generally is, in the two successive cells divisions which give rise to the polar cells. He denies that the polar cells represent male elements (Minot and Balfour), but maintains that more

than a mere reduction in nuclear mass (Strasburger) has to be attained. He asserts the probability of their general occurrence, and on à priori grounds even in parthenogenetic ova, while he has, as we have noted, lately observed a polar cell in the summer eggs of certain Daphnoids. He further applies his theory of the two kinds of nuclear plasma, and the necessary extrusion of some of the non-germinal or histogenetic, to spermatogenesis and to differentiating processes in plants.

II. Follicular Cells.

Till within the last few years it has been the all but undisputed opinion that the follicular cells, which so frequently envelop the ovum, originated entirely from outside, from adjacent germinal or non-germinal cells. Lately, however, it has been vigorously maintained that, in some cases at least, the follicular cells arise within the ovum itself, and, according to most of the supporters of this view, from the germinal vesicle.

In a too-much overlooked research (1877) on the follicular and other cells of Tunicate ova, about which so much difference of opinion has prevailed, Fol clearly distinguished the granular, hardly cellular globules, doubly misnamed "test cells," from the nucleated definite follicular cells, described the latter in contact with the germinal vesicle and half way out towards the periphery, and maintained that they really originated from the nucleus and migrated outwards. Lubbock had, indeed, long before (1861), described a budding of the nucleus, and various observations, such as that of the presence of follicular cell-like bodies in the vitellus, may be capable of interpretation in harmony with Fol's theory, but for definite statement and observation as to the origin of these cells he undoubtedly deserves the credit of priority.

In 1880 Nussbaum asserted, for the first time, the origin of Vertebrate (Amphibia and Teleostei) follicular cells from a morula-like division of the germinal vesicle, followed by a centrifugal migration of the daughter nuclei. According to Sabatier, Cadiat described in 1881 the origin of the follicular

cells within, but near the periphery of the ovum. Some observations, such as those of Schäfer and Bambeke, may also be interpreted in harmony with Fol's theory, but it was not till 1883 that attention was emphatically recalled to the subject.

Following up his previous research, Fol distinguished in the ovum of Tunicata (1) granular globules encrusted on the vitellus; (2) a thin "chorion" membrane; (3) a layer of papillary "spumeuses" cells; (4) an outermost envelope of flattened pavement-like follicular cells. Only the two outer layers are distinctly cellular, and result from a migration outwards of cells formed endogenously within the ovum, and with the distinct participation of the germinal vesicle. The endogenous formation begins by a local thickening of the nuclear membrane, the thickened portion is pressed outwards, and the nucleolus, which is generally near, seems to yield a little of its substance to the protrusion. The latter soon becomes a solid button-like bud, is attached to the nucleus by a distinct stalk, but being liberated migrates thence outwards, and forms, with associated protoplasm, a follicular cell. Fol describes these budded daughter-nuclei as appearing sometimes in succession, and then the nucleolus of the germinal vesicle remains visible, or as being formed in numbers at once with the disappearance of the nucleolus. The first set of migrant nuclei form the thin flat outermost layer, the so-called papillary cells are next formed, while a third set of migrating elements, not, however, true cells nor arising from the germinal vesicle, form the granular globules or so-called test-cells.

Sabatier, on the other hand, maintained that though the follicular cells of Tunicata were intravitelline and not from outside, yet they arose from aggregations of chromatin in the vitellus, near the germinal vesicle, but not from it. He describes them as protoplasmic concentrations, at first clear and homogeneous, but becoming differentiated into cells with distinct nuclei. The granular or "test" cells are also intravitelline eliminations, but they degenerate before their cellular differentiation is accomplished. Between follicular cells and

granular cells there is thus no radical difference; they are formed in the same region of the ovum, and in the same way, but at different epochs in ovogenesis, and with unequal cellular differentiation. Sabatier asserted, further, that in the ova of fish, amphibia, cat, dog, cow, and homo, bodies are eliminated from the germinal vesicle, which are destined to become the cells of the Graaffian follicle, but which are, as in Tunicata, formed without the participation of the germinal vesicle. Fol has also observed in frog, triton, rabbit, cat, and once in homo, phenomena which he could only explain on the supposition of migration of nuclear elements from the germinal vesicle, though, in the absence of decisive proof, he adds, "I can hardly believe that these cells, endogenously formed within the ova, could form all the epithelial envelope of the Graafian follicle. Sarasin ('Biol. Centblt.,' iii, 4) has also described Schäfer's pseudo-nuclei in the Reptilian ovum.

In Phallusia Roule described the origin of adventitious nucleoli within the germinal vesicle. These migrate into the vitellus, become surrounded by a clear zone of protoplasm, travel to the periphery, and form first the follicular, and, at a later stage, the "test" cells. The follicular envelope increases by the division of the cells which have migrated outwards. The formation of nucleoli described by Roule and the budding described by Fol are emphatically contradicted by Sabatier. In the oogenesis of Appendicularia Bolles Lee described nuclear budding of the primitive nuclei of the ovarian portion of the ovotestis, and the liberation of the buds to become on the one hand definite ova, and on the other the epithelial cells enveloping these. Balbiani next described in Geophilus phenomena which corroborated Fol and Roule rather than Sabatier. The nuclear filament of the germinal vesicle breaks up into rounded-off portions, which find their way outwards; in some cases a sort of stolon was prolonged out from the nucleus, in others the budding took place all round. The detached buds travel outwards to the periphery, and the follicular epithelium is thus gradually formed. The final germinal vesicle is simply a follicular cell, which does not travel out-

wards, but remains in the bosom of the vitellus. Sabatier ('Comptes rendus,' 1883) has also observed a yolk nucleus in the arachnid ovum, originating near or even in contact with the germinal vesicle, but not from it, a male centrifugal element breaking up at the periphery.

A recent important contribution is due to Will, whose researches relate for the most part to insect ova. He describes the morula-like division of the primitive nucleus or ooblast, the appearance in some cases of buds covering the whole surface of the germinal vesicle, and the migration of the daughter-nuclei to the periphery, leaving, however, a residue which forms the final germinal vesicle, often at first with but little chromatin left. The migrating daughter-nuclei seem sometimes simply to form part of the yolk, in other cases they result first in distinct epithelial cells, which afterwards share in the yolk formation. He has also described how the nuclei of the "nutritive cells," which result from the early division of a generative cell, may either dissolve away and help to form nutriment outside the ovum, or may wander out of their cells, and, reaching the ovum, also form epithelial cells, and thus also share in the formation of yolk.

Many of these observations must await further confirmation, but the fact that Fol, Nussbaum, Sabatier, Roule, Balbiani, Will, and others agree in deriving the follicular cells from the ovum itself, and all of those cited, with the exception of Sabatier, from the germinal vesicle, warrants some confidence in results so suggestive, both in themselves and in relation to spermatogenesis and polar cells.

I. Polar Globules and Extrusions from Reproductive Cells.

(1) Weismann.—'Die Continuität des Keimplasmas,' 1885, chap. ii, "Die Bedeutung der Richtungskörperchen," *vide* Nachschrift.

(2) Balbiani.—"Contribution à l'Étude de la Formation des Organes Sexuels chez les Insectes," 'Rec. Zool. Suisse.,' T. ii, 1885.

(3) Sabatier.—"Contribution à l'Étude des Globules polaires et des elements eliminées de l'œuf en general" (Theorie de la sexualité), Montpellier, 1884.

(4) Strasburger.—'Neue Untersuchungen über den Befruchtungsvorgang bei den Phanerogamen als Grundlage für eine Theorie der Zeugung,' Jena, 1884.

(5) Ed. v. Beneden and Ch. Julin.—"La spermatogenèse chez l'ascaride megalocephala," 'Bull de l'Acad. roy. de Belgique,' 3me sér, t. vii, 1884.

(6) Bütschli.—'Zeitsch. f. wiss. Zool.,' xxix, 1877. "Morphologische Bedeutung der Richtungskörperchen," 'Biol. Centblt.,' iv, 1, 1884.

(7) Minot.—'Proc. Boston Soc. Nat. Hist.,' xix, 1877. 'American Naturalist,' 1880, vol. xiv. "Theorie der Genoblasten" 'Biol. Cent.,' i, 12, 1882.

(From these, especially from Sabatier, a fuller bibliography may be obtained.)

II. Follicular Cells.

N.B.—A full bibliography may be obtained from the papers of Fol, Sabatier, and Will.

(1) Fol, 1877.—'Journal de Micrographie de Pelleton.'

(2) Nussbaum, 1880.—"Differenzirung des Geschlechts im Thierreich," 'Archiv f. mikr. Anat.,' xviii.

(3) Cadiat, 1881.—'Traité d'Anatomie générale.'

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The Preparation of the Eye for Histological Examination.

By

James W. Barrett, M.B.,
Demonstrator of Physiology in King's College, London.

During the last eighteen months I have been endeavouring to prepare satisfactory sections of different portions of the eye, and the following communication, which treats only of method, is based upon the results obtained.

I am especially anxious that no statement in it shall be regarded as being final, since I feel convinced that success in this as in other branches of histology depends as much on the histologist's knowledge of a method as on its intrinsic merits. My results may, however, serve to indicate the direction in which success is to be sought.

The Preparation of Sections of the Entire Eye.

I do not think that sections of an entire eye can be prepared without the aid of embedding and infiltrating materials, and I have been successful with only two, celloidin and paraffin.

1. Preparation of sections of entire eyes by infiltrating and embedding in celloidin.

The eye, removed from the body as soon as possible after death, should be opened by a short incision through the sclerotic, midway between the cornea and the entrance of the optic nerve, and should then be placed in some fixing and hardening agent (Müller's fluid, chromic acid solution). Ulti-

mately, following the usual plan, it should be transferred, first to weak, and subsequently to strong alcohol. Much of the success of the process is dependent on the care taken in fixing and hardening. Although any of the chromic acid preparations harden very well indeed, yet there are two important objections to their indiscriminate use as fixing or hardening agents,—they usually render the lens brittle and unnecessarily hard, and they often render the sections difficult to stain. Therefore, another fixing agent has been employed when I have wished to prepare sections of the lens or to stain the eye thoroughly. This agent is carbolic acid. The eyes after removal from the body should be placed in a 2 per cent. watery solution for a week, and should then be transferred to alcohol and be treated as in the former case. When prepared in this way the eyes stain readily and the lens is not usually brittle.

If a section of the eye without the lens is required it is better to use a chromic acid hardening solution, because that reagent hardens so satisfactorily.

The eye hardened by any of these methods should be stained in bulk; this is not absolutely necessary, for sections may be stained after they have been cut, but staining in bulk enables one to avoid a certain amount of dangerous after-manipulation.

Before placing the eye in the stain four small openings should be made in it, two into the anterior chamber, and two into the vitreous. The openings into the anterior chamber should be situated opposite to one another, and at the periphery; those into the vitreous should also be opposite one another, and should be situated midway between the cornea and the entrance of the optic nerve.

The only stain which was found to be reliable for staining in bulk was borax carmine (alcoholic). Kleinenberg's logwood will not penetrate sufficiently, and (in my hands) often fails to select; and most of the aniline stains (which penetrate admirably) are partially removed during the necessary after-treatment.

The eye should be left in the stain for from two to four days,

according to the rapidity with which it stains ; the staining is somewhat diffuse, and it is sometimes preferable to place the eye after staining in alcohol containing a trace of HCl in order to remove the stain from everything but the nuclei.

Formula for Alcoholic Borax Carmine (Woodward's).

Carmine, Nr. 40, gr. xv;
Borax ʒj ;
Water to 8 oz.

Dissolve by warming and slowly evaporate to 4 oz. ; now add 7 oz. of alcohol.

If it is to be used for staining in bulk there is no need to filter it. It should be shaken well from time to time.

After the eye is stained it should be washed and transferred to alcohol, and then to a mixture of alcohol and ether equal parts. In this mixture the eye should be left for twenty-four hours, when it should be transferred to a thin solution of celloidin in equal parts of absolute alcohol and ether ; an accurate measurement of the quantities of alcohol and ether is unnecessary, but the quantity of ether should never be greater than the quantity of alcohol.

In this solution the eye may be left for two or three days until the celloidin has fairly penetrated all parts of it.

Embedding.—The infiltrated eye should be placed in a pill-box or paper boat with a perfectly flat floor, and a tolerably thick solution of celloidin should be poured into the box until the eye is completely covered. The box or boat should then be placed on a glass plate and should be covered with a bell jar ; the alcohol and ether diffuse into the air beneath the bell jar and the celloidin slowly consolidates. If a bell jar is not used a crust usually forms on the surface of the celloidin and further evaporation is hindered, whilst on the other hand the use of the bell jar permits of an equable removal of the alcohol and ether from all parts of the mass without the formation of bubbles. It should be lifted from time to time to allow of a partial removal of the gaseous alcohol and ether. The use of the bell jar is

particularly indicated in hot weather, and when the mass of celloidin is very large.

When the mass becomes tolerably firm it should be transferred to a mixture of equal parts of commercial alcohol and water, in which the consolidation soon becomes complete. The time the mass must be left under the bell jar depends much on the temperature of the room, and varies from one to six days.

The eye is now infiltrated with and firmly embedded in celloidin, and sections of it may be prepared.

It is almost impossible to cut sections from the block of celloidin in this condition on account of its size, so the whole mass should be cut into slices about a quarter of an inch thick, and one of these pieces should be fixed in the microtome. If the division into slices be made before embedding the lens will be displaced.

When the eyes are exceedingly large, and embedding is consequently difficult, I usually re-embed one of these slices, and so obtain a requisite degree of hardness.

Sections may be cut in three ways:

a. By the freezing microtome.

b. By any "slide microtome," such as Jung's.

c. By a microtome so arranged that the section may be cut under spirit.

(*a*) The mass should be placed in water for from six to twenty-four hours, until the greater part of the spirit has been removed. It should then be dipped in gum for a moment, and may be frozen, the gum serving to attach it to the plate of the microtome.

Sections may next be cut and should be floated off the knife into warm water. If all the spirit has been removed from the mass, the celloidin, when frozen, often becomes intensely hard and difficult to cut. This difficulty may readily be obviated by warming the knife in warm water before cutting the sections.

(*b* or *c*) The mass should be securely fixed to a cork-covered plate. This is always difficult to do unless there is one flat

surface to the celloidin. The most rapid method of fixing is to moisten the cork and the flat surface of celloidin with ether, and to firmly press the moistened surfaces against one another for five to ten minutes; the ether has then evaporated, and the celloidin firmly adheres to the cork.

Another method is to smear some thick solution of celloidin over both surfaces, to press them together for fifteen to thirty minutes, and then to place them in alcohol for twenty-four hours.

There are also other methods of securing the mass, with gelatine or with paraffin, but the two methods described are rather more simple. As regards the relative values of the three methods of section-cutting I think that for the preparation of sections of small pieces of the eye the freezing method answers well, and has the merit of being very convenient, but for the preparation of sections of pieces of any size the method of cutting under spirit is most suitable.

When the sections have been prepared they may be stained or simply mounted; they should always be manipulated between two pieces of tissue paper, since any rough usage causes displacements. They must be thoroughly dehydrated by long immersion in alcohol, and may be then cleared in one of three media:

a. Oil of bergamot.

b. Oil of cedar.

c. Turpentine.

Of these oil of bergamot acts the most rapidly and efficiently; at times, however, samples of oil of bergamot are met with which will dissolve celloidin.

Oil of cedar is very slow in its action; and turpentine often causes a disagreeable shrinkage.

The sections should be mounted in balsam.

I have obtained sections by the freezing method, which are fairly good histological specimens, and which will bear examination with a high power; but by the other method I have rarely obtained sections which serve to illustrate more than the topographical anatomy of the eye—sections which may be examined with a half-inch objective—although, if the

eye be that of a very small animal, the result has been sometimes better.

I have further found that this process almost always produces some histological changes in the tissues; they are, however, sometimes slight.

Infiltration with Paraffin.—This method is exceedingly useful for the preparation of sections of the eyes of embryos, of the eyes of very young animals, and of sections of eyes in those cases where the examination of the lens is not necessary. Its great merit lies in its simplicity.

I have practised two methods of infiltrating. The turpentine process:

a. The eyes hardened, opened, and stained, as before, are transferred from alcohol to oil of cloves, in which they are left until they are cleared; they are then soaked in pure turpentine for several hours, and are finally placed for twelve to forty-eight hours in paraffin, melted at a temperature not exceeding 50° C. The paraffin displaces the turpentine and permeates the crevices of the tissue. The infiltrated eye is then embedded in paraffin, and sections may be cut and sealed to the slide in the usual manner. The cement which has been most serviceable to me is a mixture of oil of cloves and collodion.

It is practically impossible to stain the sections after they have been cut and sealed to the slide. Certain passable results may occasionally be obtained by the use of diffusible stains, but as a rule the result is disappointing.

Unfortunately, this process nearly always ends in the total histological destruction of the lens (in fact too often a section of the lens cannot be prepared, since it instantly crumbles to pieces), and too frequently renders the tissues unfit for very minute examination. I thought that this alteration of the tissues was due to the high temperature of the melted paraffin, and I therefore obtained a paraffin which melted at 35° C., infiltrated the eyes with it, and then embedded in a harder sample. The tissue was, nevertheless, somewhat altered. I have, however, obtained better results by using the paraffin in a different manner.

b. After staining, the eye is placed in a mixture of alcohol and ether, equal parts, for twenty-four hours, and is then submersed in pure chloroform for two days. It is finally placed in melted paraffin for twelve to forty-eight hours, and is treated subsequently as in the former case. By the use of chloroform the treatment with turpentine and oil of cloves is avoided.

Conclusions.—1. Satisfactory sections of a small portion of the eye may be easily obtained by infiltrating and embedding in celloidin, and by cutting sections either with the freezing microtome or under spirit. Such sections may, if necessary, be stained after they have been cut.

2. Sections of parts of the eye without the lens of young or of embryonic eyes may be readily obtained by infiltrating and embedding in paraffin by the chloroform process. The eye must in this case be stained in bulk.

3. Sections of the eye with the lens in sitû may be best procured by infiltrating and embedding in celloidin and cutting under spirit.

If sections of the Classes 1 and 2 are required I believe that it is better to harden the eyes in chromic acid, but if sections of Class 3 are in demand the fixation and hardening should be effected by the use of alcohol and carbolic acid.

Preparation of Retina.

When I first endeavoured to prepare sections of retina I had to determine :—

a. The best fixing and hardening agent.

b. The best staining agent.

c. The best embedding agent.

(*a*) I obtained many eyes from guinea-pigs, fixed and hardened them in different solutions, and prepared sections of the retina. But except in the matter of hardening all were prepared in the same way, so that in the fixing and hardening the only variable factor was consciously introduced.

The sections were prepared in the first set of experiments by infiltrating and embedding in celloidin in the manner already described.

The following fixing and hardening solutions were employed:

1. Müller's Fluid.—The fresh eye, opened in the way already described, was placed in Müller's fluid for two or three weeks, during which time the fluid was changed as often as its altered appearance afforded an indication of the necessity. It was then transferred, after being washed, to strong commercial alcohol, and was completely hardened in about two weeks. Sections of retinas so prepared were serviceable in showing the structure of the inner layers of the retina and the course taken by the blood-vessels (in retinas which contain them), but the rod-and-cone layer and the outer nuclear layer were more or less completely destroyed.

2. Bichloride of Mercury.—A saturated watery solution was employed; the freshly opened eye was placed in this solution for three to six days, and was then hardened in alcohol as before. Some eyes I placed in alcohol containing 2 per cent. of carbolic acid instead of simple alcohol.

The salt "fixed" in a manner much superior to Müller's fluid, but usually permitted or caused shrinkage in the rod layer.

The sections of retinas prepared with the alcoholic solution of carbolic acid were superior histologically to those prepared in alcohol alone, and this I found to hold good for all the fixing agents employed.

It occured to me at this stage of my work that possibly the fixing solution did not gain access to the retina with sufficient rapidity, the opening in the eye not being large enough; yet a very large opening allows the retina to become detached. I therefore procured two cannulæ, and pushed them through the coats of the eye into the vitreous at points a little distant from one another; then I endeavoured to fill the vitreous with the fixing agent by injecting it through one of these cannulæ whilst the intraocular tension remained unaltered.

No good results followed, chiefly because of the firm consistency of the vitreous. A more simple method was then adopted; the length of the incision was made equal to a quarter of the circumference of the eye, and the eye was then placed in the fixing solution. At the end of thirty minutes or less the posterior part of the eye was removed by enlarging the original incision with sharp scissors. By this means the fixing agent obtained access to the retina rapidly, and detachment of the retina was prevented.

3. Picric Acid.—The fresh and opened eye was placed in a saturated watery solution of picric acid for three days, and the hardening was then completed in alcohol and carbolic acid. By this fixing agent everything was rendered intensely hard but rather brittle. Sections of retina prepared in this way were very serviceable in showing the structure of the nerve-layers of the retina, but the outer nuclear layer and the rod layer were profoundly altered. The nuclei (with a twelfth oil immersion lens) showed a remarkable crenation, whilst similar nuclei in another eye prepared with such a reagent as osmic acid showed no such crenation. By the use of picric acid, however, it was possible to trace the Müllerian fibres at all events as far as the outer reticular layer, since the previous immersion of the retina in picric acid seems to intensify the eosinophilous property which those fibres exhibit.

4. Carbolic Acid.—The fresh eye, prepared as before, was placed in a 2 per cent. watery solution of carbolic acid for a week, and was then hardened in alcohol in the usual manner. Carbolic acid itself does not harden. By this means fair specimens of all parts of the retina were occasionally obtained.

5. Zinc Chloride.—The fresh and opened eye was placed for a week in a 1 per cent. watery solution of this salt and was then removed to the alcoholic solution of carbolic acid. The zinc salt did not harden, and seemed to destroy the outer layers of the retina, but its action on the Müllerian fibres was similar to that of picric acid.

6. Permanganate of Potash.—The fresh and opened eye was placed in a 2 per cent. solution of this salt for seven days

and was then hardened in alcohol and carbolic acid. The permanganate salt did not harden, and the sections of retina prepared in this way were unsatisfactory.

7. Chromic Acid.—The fresh and opened eye was placed in a $\frac{1}{6}$ per cent. watery solution of chromic acid and was allowed to remain there for twenty-four to forty-eight hours. The hardening was then completed by the use of the alcohol and carbolic acid solution. If the eye was left more than forty-eight hours in the chromic acid solution difficulty was experienced both in staining and in the preparation of sections (on account of brittleness). Sections so prepared were usually very serviceable in showing the structure of all the layers except the rod layer.

8. Chloral Hydrate.—The fresh and opened eye was placed in a 10 per cent. solution of this salt for two to seven days; the hardening was completed by the alcohol and carbolic solution. Chloral did not harden, and in my hands only yielded first-class results occasionally. It certainly has the merit of preserving the rod layer, and it is quite possible by this method to obtain satisfactory specimens with the rods and cones in situ.

9. Chloride of Gold.—I have made very many efforts to obtain sections stained with this salt, but they have not been successful.

The following methods have been employed:

a. The fresh freely-opened eye was placed in a solution of 1 per cent. of the salt for fifteen to forty-five minutes and was then transferred to a weak solution of formic or acetic acids, and was left there in the dark till the salt was reduced (usually twenty-four to forty-eight hours).

b. The fresh freely-opened eye was placed for one to three minutes in weak formic acid, and was then treated as before.

c. The fresh freely-opened eye was placed for several days in a $\frac{1}{6}$ per cent. watery solution of chromic acid. When hardened the eye was placed in a neutral or slightly alkaline solution of the gold salt for thirty minutes and was transferred to a solution of weak formic acid kept at a temperature of 30° C. in the

dark. At the end of twenty-four hours the reduction was complete. This process is a modification of that which Mr. Underwood employs with great success in the preparation of sections of teeth.

d. The fresh freely-opened eye was placed in a solution of osmic and chromic acids (afterwards described) for two to five days and was then treated as in *c.* By none of these methods have I been able to procure one satisfactory section.

10. Osmic Acid.—By means of this very reliable reagent I have obtained my best results.

a. The fresh and opened eye was placed for twenty-four to forty-eight hours (not longer) in a watery solution of osmic and chromic acids; $\frac{1}{4}$ per cent. chromic acid, $\frac{1}{10}$ per cent. osmic acid. It was then placed in the mixture of alcohol and carbolic acids for fourteen days or more. By this process the retina was rendered exceedingly hard but not brittle. The sections showed the structure of all parts of the retina, the rods being sharply defined and remaining in sitû. (One of these sections was exhibited at the December meeting of the Physiological Society, 1885.)[1] If the retina was allowed to remain in the solution for more than forty-eight hours brittleness was usually produced.

b. The fresh and opened eye was placed in a ·75 to 1 p. c. solution of osmic acid for from thirty minutes to twelve hours, and was subsequently treated with (*a*) alcohol, glycerine and water, or (*b*) alcohol, or (*c*) alcohol and carbolic acid. The hardening was not usually good and the results were often only passable.

c. In order to obtain very rapid penetration of the retina by the fixing agent, solutions of osmic and chromic acid in alcohol were employed.

They were:

1.	Osmic acid .	$\frac{1}{10}$ per cent.
	Chromic acid .	$\frac{1}{4}$,,
	Commercial alcohol, Water,	Equal parts.

[1] 'Proceedings Physiological Society,' December, 1885.

2. Osmic acid	. $\frac{1}{5}$ per cent.	
Chromic acid	. $\frac{1}{6}$,,	
Commercial alcohol,		Equal parts.
Water,		

With these solutions the layers of the retina, exclusive of the rod layer, were very fairly prepared, but in that layer shrinkage was produced.

d. The fresh and opened eye was placed in a solution of

Osmic acid .	. $\frac{1}{5}$ per cent.
Chromic acid	. $\frac{1}{6}$,,
Water.	

for twenty-four to thirty-six hours, and was then transferred to the alcohol and carbolic solution and treated as before. By this method the most uniform and certain results have been obtained. All parts of the retina were fixed and preserved in a manner superior to that produced by any of the other reagents used.

Mode of Staining.—It is quite possible to stain sections of retina if they have been prepared by the celloidin method, but if they are to be prepared by the paraffin or cacao butter method, the retina must be stained in bulk before it is embedded (at least with the nuclear stain). Two nuclear stains, logwood and carmine, have been chiefly used, there being objections to the use of the anilines. Kleinenberg's logwood and the alcoholic borax carmine already described were selected; if thick sections are required (as in searching for blood-vessels) the carmine is preferable because it is a transparent stain, whilst if the thinnest sections are required nothing equals Kleinenberg's logwood.

Retinas should be left in the carmine about two days and in the logwood from twelve to twenty-four hours. The exact time depends much on the hardening agent which has been used, and must vary for each retina. If only very thin sections be cut a moderate amount of overstaining with logwood does no harm whatever.

In order to examine Müllerian fibres or blood-vessels the sections of the retina which have already been stained in bulk

with a nuclear stain should be stained (best on the slide) with either fuchsin or an alcoholic solution of eosin, preferably the latter. The exact method of staining will be described.

Mode of Preparing and Mounting Sections.—Sections may be obtained by :

1. Infiltrating and embedding in celloidin and freezing.

2. Infiltrating and embedding in celloidin and cutting under spirit.

3. Infiltrating and embedding in paraffin.

4. Infiltrating and embedding in cacao butter.

1 and 2. It is difficult to obtain thin sections by the second method, but very fair ones may be obtained by—(1) the whole sclerotic, choroid and retina should be embedded together, and when the celloidin is firm the retina and part of the choroid should be separated with a sharp scalpel; attempts to separate the retina earlier generally end in damage to the rod layer. After the sections have been cut by the method previously described they may be diffusely stained and mounted. The staining may be effected in two ways : (*a*) to the water in which the sections have been placed on removal from the microtome a little eosin is added; in a few minutes they will be sufficiently stained; or (*b*), they may be at once placed on the slide with a section lifter and the staining may be effected there. In either case after staining they should be gently washed and nearly dried with blotting paper, then covered with a few drops of alcohol. On removing this reagent with blotting paper they should be cleared either in oil of cloves or oil of bergamot and may be mounted in balsam.

3. By the paraffin method already described serial sections may be prepared, but I have never yet obtained by this method any sections of very great histological value; they have been at best passable.

4. Infiltrating and embedding in cacao butter. By this method I have been able to prepare the thinnest and best sections of retina with a minimum amount of trouble. A piece of the eye stained with a nuclear stain should be placed first in alcohol until dehydrated, then in oil of cloves till

cleared, and then in cacao butter melted at a temperature of 35° C. for four to six or even twelve hours. At the end of this time it should be embedded in cacao butter in the usual manner. When the butter is quite hard, the sclerotic and part of the choroid should be detached with a sharp scalpel so that the retina and part of the choroid alone remain to be cut into sections, whilst the rod layer has never been tampered with.

The retina should be fixed by pouring over it a little more melted butter which replaces the mass cut away.

The sections may be cut either by hand, or with any accurately constructed "slide microtome," and with care may be made only one nucleus in thickness. Such sections are nearly invisible to the naked eye. If a microtome is used and such sections are prepared, they accumulate on the blade of the knife and look like a little mass of butter. This mass should be swept on to a slide, when the contained sections may be diffusely stained and mounted in the following manner:

A few drops of an alcoholic solution of eosine are poured over the mass and at once soak into it; after a few minutes the mass is partially dried with blotting paper, and the slide is heated to a temperature of 35° C. The melted cacao butter is removed as far as possible with blotting paper, and a drop of oil of cloves is added to remove the remainder. When the sections are cleared a drop of balsam is added and the sections are mounted.

It is very important to remove as much butter as possible before adding the oil, because the oil acts very violently and often destroys a section. In fact the great value of osmic and chromic acids as hardening agents depends largely on the great hardness they give to the retina, the sections of which are therefore not damaged by the oil of cloves.

Conclusion.—I have been able to prepare the best sections of retina by fixing and hardening in the watery solution of osmic and chromic acids in the manner described, staining in bulk with Kleinenberg's logwood and infiltrating and embedding in cacao butter.

Finally, I desire to acknowledge with sincere thanks the

assistance which has been afforded to me by Professor Fuchs, of Vienna (late Liége), by Dr. Bäumler, of Halle, and by Dr. Gade, of Kristiania; also by Mr. Gunn, of Moorfields Eye Hospital, and by Mr. E. F. Herroun, of King's College, London.

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